'The Neurochemistry of Huntington's Disease'

by Sally Jane Pearson, B.Sc.

Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy, May, 1992

ABSTRACT

This thesis describes the study of the neurochemistry of Huntington's disease using a large series of post mortem brain tissue taken from patients with Huntington's disease and from matching controls with no previous history of neuropsychiatric disorder. There were two main aims: firstly, to identify and characterise any altered parameters of neurotransmitter systems, especially in relation to the symptomatology of the disease; secondly, to understand the role of neurotoxins in the aetiology of the disease, particularly endogenous compounds that may have derived from aberrant metabolism.

Concentrations of the amino acid transmitters, GABA and glutamate, were generally significantly decreased throughout the brain in Huntington's disease, including cortical and limbic regions. Cortical deficits were not associated with the dementia of the disease, whereas caudate levels of GABA and glutamate showed a relationship with the dementia. In patients with severe chorea, the medial pallidum was found to have a relatively smaller GABA deficit than mildly choreic patients. Another novel finding was that 5HT and 5HIAA concentrations were significantly increased in most regions of the brain in Huntington's disease, perhaps reflecting abnormal tryptophan metabolism. Such changes in the cortex provide evidence for a cortical involvement in the disease. Dopamine metabolism appeared to be reduced in Huntington's disease, reflected by the significantly decreased concentrations of its major metabolite, homovanillic acid, in most regions except for the cortex (where it was increased).

Neuroactive compounds of the kynurenine pathway of tryptophan metabolism were measured in Huntington's disease. Quinolinic acid concentrations were not significantly altered, however 3-hydroxykynurenine concentrations were significantly increased in the striatum and cortex. This provides the first evidence for increased concentrations of an endogenous neurotoxic compound in the brain in Huntington's disease.

Tables

Figures

Abbreviations

BABA	ß-aminobutyric acid
GABA	γ-aminobutyric acid
GABA-T	γ-aminobutyric acid transaminase
ACE	acetylcholinesterase
ACEZ	angiotensin-converting enzyme
ACH	acetylcholine
ACMS	α-amino-ß-carboxy-δ-semialdehyde
AD	Alzheimer's disease
AP	2-amino-phosphonic acid
AZT	zidovudine
BA	Brodmann's area
CAT	choline acetyltransferase
CCK	cholecystokinin
CI	chemical ionization
4-Cl-3HA	4-chloro-3-hydroxyanthranilic acid
CSF	cerebrospinal fluid
CT	computed tomography
DA	dopamine
DHBA	dihydroxybenzylamine
DTG	1,3-di-o-tolyl-guanidine
ECF	extracellular fluid
ED	electrochemical detection
EDTA	diaminoethanetetra-acetic acid
EI	electron impact
GAD	glutamic acid decarboxylase
GA-I	glutaric aciduria-type I
GC-FID	gas chromatography-flame ionisation detection
GC-MS	gas chromatography-mass spectrometry
GI	GTP insensitive
GS	GTP sensitive
3HA	3-hydroxyanthranilic acid
3HAO	3-hydroxyanthranilic acid oxygenase
HCA	homocysteic acid
HCl	hydrochloric acid
HD	Huntington's disease

HFIP	1,1,1,3,3,3-hexafluoroisopropanol
5HIAA	5-hydroxyindoleacetic acid
3HK	3-hydroxykynurenine
HPLC	high-performance liquid chromatography
5HT	5-hydroxytryptamine
5HTP	5-hydroxytryptophan
HVA	homovanillic acid
i.p.	intraperitoneal
IS	internal standard
KA	kynurenic acid
MAO	monoamine oxidase
MRI	magnetic resonance imaging
NA	noradrenaline
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NADPH-d	reduced nicotinamide adenine dinucleotide phosphate diaphorase
NAMN	nicotinic acid mononucleotide
NMDA	N-methyl-D-aspartate
NPY	neuropeptide Y
OPA	o-phthalaldehyde
PA	portocaval anastomosis
PB	phosphate buffer
PBS	phosphate-buffered saline
PCA	perchloric acid
PCP	phencyclidine
PD	Parkinson's disease
PDA	piperidine dicarboxylic acid
PET	positron emission tomography
PFA	pentafluoropropronic anhydride
PGA	pyroglutamate
PIA	phenylisopropyladenosine
+3PPP	+3-(3-hydroxyphenyl)-N-(1-propyl) piperidine
QA	quinolinic acid

QPRT	quinolinic phosphoribosyltransferase
SAX	strong anion exchange
SCX	strong cation exchange
s.nigra	substantia nigra
SOD	superoxide dismutase
SPECT	single photon emission computed tomography
SS	somatostatin
STN	subthalamic nucleus
TFAA	trifluoroacetic anhydride
TPB	tetraphenylboron
TRH	thyrotropin-releasing hormone
TRIS	tris (hydroxymethyl) methylamine
VA	venteroanterior
VIP	vasoactive intestinal peptide
VL	venterolateral

Acknowledgements

Firstly, I am immensely grateful to my supervisor, Dr Gavin Reynolds for his erudite guidance and infinite patience; providing endless encouragement, with only the occasional necessary nagging.

Secondly, thanks are due to the Huntington's Disease Association who have generously supported this study for several years from their limited research funds.

Facilities of the Pathology Department (Queen's Medical Centre, Nottingham) were provided courtesy of Professor David Turner.

Next, I would like to thank the staff at the Cambridge Brain Bank for cheerfully answering incessant pleas for ever more tissue. It is also necessary to thank Dr L Carrasco for neuropathological assessment of the brains and Dr K Heathfield for providing the clinical assessments of the cases used in this study.

The assistance of Dr John Halket and Professor Merton Sandler (Queen Charlotte's Hospital, London) is greatly appreciated, enabling gas chromatograph-mass spectrometric analyses to be made.

Furthermore, I am obliged to Margaret Johnson and Dr E Blank (Medical Genetics, Sheffield) who provided the blood samples from patients with Huntington's disease.

Clearly, I must recognise the spirited support of my colleagues and friends in Nottingham, Beverley and Sheffield. In particular, Carole Czudek (my role model), Jane Jones, Malcolm Bloomfield, Andy et al. (my laboratory comrades in D58), The Rashids, Carl and Co. and Maurice Sanders (who deserves a special mention for leading me astray and keeping me sane).

Finally, this study could not have been made without the generous brain tissue donation from families facing the everyday challenges of Huntington's disease (and especially my own family).

Chapter 1

Introduction

1.1 General background information

In 1872 George Huntington, from New York, published the first medical description of the disorder, Huntington's disease (HD): 'On Chorea' (Huntington, 1872). HD is found throughout the world, but there is evidence suggesting early transmitters of the HD gene emigrated from Bures, East Anglia to the USA in 1630.

HD is a progressive autosomal dominant disorder of unknown aetiology with 100% penetrance. DNA markers linked to the gene have been localised to the short arm of chromosome 4 (Gusella et al., 1983), but as yet the locus of the gene itself remains unknown. Recombinant DNA technology and linkage analysis have enabled predictive and prenatal testing to be developed, now available for those at risk of inheriting the disease, but currently the accuracy is only about 95% (Wexler et al., 1991). New mutations are thought to be unlikely (Martin, 1984) and there are no proven cases. The prevalence of HD varies between areas, but 7.5 per 100,000 is an average figure for England (Hayden, 1981). The onset of the disease is usually in the 3rd-4th decade with an average duration of 15-20 years. However, onset can occur at any age, although the character of the disease may be different. Juvenile HD (onset < 20 years) often displays a more severe and rapid progression whereas, when the onset is late in life (onset > 60 years), the progression is milder and slower. A further notable effect is 'anticipation'. Patients inheriting the HD gene from the father have been shown to have onset 10

years earlier than their parent (Bird, 1974). Furthermore, studies of juvenile HD demonstrate that they are four times as likely to have inherited the disease from the father, than from the mother (Barbeau, 1970). Thus, a sex-associated modifying gene may be influencing the HD gene. Homozygotes have been reported to be phenotypically similar to heterozygotes (Wexler et al., 1987).

1.2 Clinical profile

Clinically, the disease is heterogeneous, characterised by the progressive movement disorder, psychiatric symptoms and dementia. Chorea (an involuntary, jerky, random hyperkinesia) is usually present in about 90% of patients, beginning slightly and becoming more pronounced as the disease progresses, but with increasingly more rigidity and akinesia in the latter stages. Some cases, such as the Westphal variant, have no chorea at all and only show rigidity; this is particularly common in juvenile HD. Other neurological symptoms include myoclonus, spasticity, bradykinesia, tremor, athetosis, dysarthria, dysphagia, olfactory dysfunction, oculomotor dysfunction (e.g. saccades) and latterly, epilepsy (Hayden, 1981). Behavioural changes may precede or appear concurrently with the early motor abnormalities. Symptoms such as unipolar depression, aggression, apathy, schizophreniform psychosis, anxiety and mania have been reported. Peyser and Folstein (1990) made a study using HD as a model for mood disorders. They described affective disorder as the most common psychiatric symptom (38%) in HD, preceding chorea in over 50% of the cases. Ten per cent also had episodes of mania, 6% schizophreniform psychosis, 31% showed severe irritability, and aggressive symptoms (59%) have been reported in a further study

by Burns et al. (1990). Cognitive impairment is a further common feature in HD, occurring in 84% of cases (Pflanz et al., 1991), but the precise nature of the dementia is controversial. Many believe it is subcortical in origin, resembling dementia in Parkinson's disease (PD) and qualitatively different to that seen in Alzheimer's disease (AD), where there is aphasia and agnosia (Chui, 1989). Cognitive symptoms in HD include forgetfulness, apathy, slowness of thought (McHugh and Folstein, 1975). Other clinical signs include excessive sweating, increased appetite and weight loss, not clearly due to increased energy demands of chorea or rigidity (Sanberg and Fibiger, 1979; Morales et al., 1989). Thyroid function has been reported to be normal (Bird and Coyle, 1986) and a possible link with diabetes mellitus (Podolsky et al., 1972) has been strongly disputed (Kremer et al., 1989). However, growth hormone regulation is reported to be disturbed and prolactin release decreased (Kremer et al., 1989; Hayden et al., 1977). Juvenile HD (6%) has a different clinical profile with increased rigidity, epilepsy, cerebellar signs and rapid progression; symptoms which may be compared with late-stage HD (Hayden, 1981).

1.3 Neuropathology

1.3.1 Macroscopic studies

Gross pathological assessment of the brain in HD show it to be generally shrunken (with a 20-30% loss in brain weight) (Bird and Spokes, 1982; Lange, 1981; De La Monte et al., 1988). There is a widening of the sulci and a reduction of the gyri and the meninges are often thickened (Hayden, 1981). Coronal sections show dilated lateral ventricles, partly as a consequence of shrinkage of the caudate

and putamen. The extent of observed tissue losses is not uniform throughout the brain, with the basal ganglia appearing to have the greatest atrophy. Lange et al. made extensive quantitative investigations of the HD brain (1976, 1981). They described volume reductions in the caudate (55% loss), putamen (57% loss), lateral pallidum (57% loss), medial pallidum (50% loss), subthalamic nucleus (24% loss) and cortex (20% loss). These values are consistent with results from a study by De La Monte et al. (1988) that also reported 20-30% loss of hippocampus, nucleus accumbens, white matter, amygdala and thalamus. Furthermore, they suggested that the 200% increase in ventricular volume was disproportionate to the shrinkage of structures around it and thus was a primary finding of hydrocephalus in HD. Other regions of known pathology in HD are substantia nigra (s.nigra) pars compacta and pars reticulata, hypothalamic nuclei (supraoptic, venteromedial, lateral tuberal, paraventricular and tuberomamillary), cerebellum, corpus callosum, pons, medulla oblongata, claustrum, tegmentum, dentate nuclei and superior olivary, vagal and hypoglossal nuclei, as well as pallor in the spinal cord (anterolateral tract) (Oyangi et al., 1989; Kremer et al., 1990; Bruyn et al., 1979; Lange et al., 1976; Vonsattel et al., 1985; Hayden, 1981). No pathology was reported in the basal nucleus of Meynert, dorsal raphé or locus coeruleus (Mann, 1989). The neuropathological involvement of the cortex in HD has provoked much discussion. Vonsattel et al. (1985), Hallervorden (1957) and Zalneraitis et al. (1981) all produced studies refuting any cortical abnormalities in HD, however, recent evidence presents a strong case for global cortical losses (20%), not correlated with striatal changes (Cudkowicz and Kowall, 1990; De La Monte et al., 1988; Bruyn et al., 1979). There is evidence

suggesting a gradient of atrophy throughout the basal ganglia in HD, which varies according to the progression of the disease. Thus Reiner et al. (1988) noted that the lateral pallidum showed atrophy much earlier than the medial pallidum, and likewise with the s.nigra (pars reticulata) compared with s.nigra (pars compacta). Furthermore, dorsal and medial parts of the caudate and putamen were found to be affected earlier than the anterior parts, parallelled by the relatively less atrophy in the nucleus accumbens until later stages of the disease process (McCaughey, 1961; Bird, 1980; Vonsattel et al., 1985). A comprehensive investigation was made by Vonsattel et al. (1985) to try to grade severity of the disease with atrophy of the caudate and putamen (by macroscopic and microscopic neuropathological criteria). The grades (0-4) were shown to correlate strongly with clinical rating. Thus, grade 1 required changes in the paraventricular caudate, tail of the caudate and dorsal putamen. These changes were demonstrated to progress laterally and basally throughout the grades, until grade 4 which displayed severe degeneration throughout the striatum. The nucleus accumbens was only found to be damaged in grade 4 cases and always relatively less affected. The pallidum was described as only shrunken in grades 3 and 4. In 3% of cases there were no neuropathological differences (grade 0), although clinical symptoms were displayed, together with a positive family history. This was suggested to be an illustration of 'anatomy lagging behind clinical manifestations'. However, there have been reports of the converse situation, in particular a case which displayed severe atrophy of the caudate and putamen, but was presymtomatic (Carrasco and Mukherji, 1986). A further case of interest showed grade 0 neuropathology, but exhibited selective

intermediate glutamatergic deficits (Albin et al., 1990b). Juvenile HD cases show faster atrophy and more severe pathology than adult cases (Reiner et al., 1988).

1.3.2 Neuronal losses

It is important to understand the actual cell types lost in HD and many studies have been made. It is evident that there are no uniform losses but, instead, specific neuronal deficits in specific regions of the brain. It has been demonstrated that within the striatum in HD, spiny projecting and interneurones are lost, whilst medium aspiny interneurones are spared. There is also an increase in the ratio of large:small neurones (Graveland et al., 1985; Ferrante et al., 1987b; Reiner et al., 1988; Lange et al., 1976). One such population of striatal interneurones that are spared have been demonstrated to contain somatostatin (SS), neuropeptide Y (NPY) and NADPH-diaphorase (NADPH-d) (Ferrante and Kowall, 1987; Dawbarn et al., 1985). This selectivity is also evident in the lateral pallidum, but not in the medial pallidum (Dawbarn, 1985). Normally, the striatum is defined by a pattern of patch or matrix zones (Graybiel and Ragsdale, 1978, 1984). The patches are believed to contain relatively reduced activity of acetylcholinesterase (ACE), tyrosine hydroxylase, as well as increased levels of enkephalin, VIP, CCK, substance P, dynorphin and opiate receptors. In contrast, the matrix is rich in ACE, tyrosine hydroxylase, calbindin, calcineurin, synaptophysin, SS, NPY and NADPH-d (Ferrante et al., 1987b). Ferrante et al. (1987b) demonstrated that the patches are relatively unaltered in HD striatum, but the matrix shows substantial deficits. Thus, in HD losses of calbindin, calcineurin and synaptophysin

occur from the medium, spiny cells of the matrix (Seto Oshima et al., 1988; Ferrante et al., 1988; Goto and Hirano, 1990). Tyrosine hydroxylase immunoreactivity remained not significantly changed despite being located in the matrix (Ferrante and Kowall, 1987); also ACE-containing large aspiny interneurones were found to be relatively spared in the matrix, as were the somatostatin/NPY/NADPH-d neurones. Furthermore, as choline acetyltransferase (CAT) activity may co-localise with ACE activity (Levey et al., 1983), it was hypothesized by Ferrante et al. (1987a) that the CAT-containing axons and terminals may be lost, whilst the same ACE-containing cell bodies survive. Whether the spines or the size or the contents of the neurones are the vital characteristic for neurodegeneration to occur remains unclear. It was previously suggested that SS/NPY/NADPH-d neurones were spared because NADPH-d had some protective detoxifying role, but this is unlikely to be the case for ACE neurones (Ferrante et al., 1987b). There was evidence that the main cortical input (glutamatergic) usually terminates on spiny cells in the matrix; these are the same neurones which are lost in HD (Ferrante et al., 1987a) and it is known that an intact corticostriatal projection is a prerequisite for neurotoxicity (Schwarcz et al., 1984b). A further hypothesis was that SS/NPY/NADPH-d neurones (and possibly ACE neurones) were resistant because they lacked NMDA receptors (Koh et al., 1986). Finally, areas rich in SS/NPY/NADPH-d neurones are those that are relatively unaffected in HD, therefore, it may be offering some kind of resistance.

In the striatum, dopamine (DA), vasopressin, 5-hydroxytryptamine (5HT) and noradrenaline (NA) afferents show no significant losses in HD.

Striatal projection neurones are differentially lost in HD. Reiner et al. (1988) described subgroups of GABAergic neurones which were co-localised with neuropeptides. Thus, the striatal projections to the s.nigra (pars reticulata and pars compacta) and to the medial pallidum contained substance P and the striatal projections to the lateral pallidum contained enkephalin. Reiner et al. also described early losses from the lateral pallidum and the s.nigra (pars reticulata) pathways in HD, compared with the medial pallidum and s.nigra (pars compacta) pathways. However, as the disease progresses there is further degeneration until all the pathways are equally affected.

A gliosis in HD has been described often (Kowall et al., 1987; Stevens et al., 1988; Martin, 1984) and Bruyn et al. (1979) has even suggested that HD could be caused by failure of glial function. However, Lange et al. (1976) reported that there is no absolute increase in numbers of glia, but in fact a decrease, and that the gliosis was relative to atrophy of the striatum. Vacca-Galloway and Nelson (1984) provided a further hypothesis: that astrogliosis may precede or contribute to the disease. The study cites evidence that abnormal astrocyte proliferation occurs in some areas such as the striatum, but not in the pallidum and therefore is not merely reflecting neuronal atrophy.

Cellular losses within the cortex are documented as occurring in layers 3, 5 and 6 (Bird and Coyle, 1986) and the atrophy appears to be general throughout the cortical regions, and unrelated to the severity grades of HD (De La Monte et al., 1988). A recent study (Cudkowicz and Kowall, 1990) identified a degeneration of frontal cortex pyramidal projection neurones in HD, which are

likely to be glutamatergic and to innervate the striatum (Oka, 1980). Cudkowicz and Kowall also found a relative sparing of NPY neurones, but there was no correlation between the cortical losses and striatal losses or grade of disease severity. However, Sotrel and Myers (1990) reported the same loss of pyramidal cells in the prefrontal cortex, but showed an association with severity of disease and no significant increase in glial cells.

Hippocampal changes specifically appear to involve neuronal losses in the pyramidal band, H5 field and subiculum (Bird and Coyle, 1986). In the s.nigra it has previously been reported that the pars reticulata was more atrophied than the pars compacta and thus appeared darker due to a relative increase in melanin and tyrosine hydroxylase containing cells (Bird and Coyle, 1986; Richardson, 1990). A morphometric study by Ferrante et al.(1989) produced data to support previous reports: the compacta showed 18% loss and the reticulata showed 68% loss. Neuronal counts showed no overall loss of pigmented neurones, but 65% loss of non-pigmented neurones. This is also consistent with observations of Waters et al. (1988) that there was no significant loss of pigmented cells in the s.nigra in HD. However, there is a contrary report (Oyanagi et al., 1989) suggesting equal losses (40%) of both pigmented and non-pigmented cells, with a relative sparing of the central part of the s.nigra in HD. Studies into the cerebellar changes in HD describe a degeneration of Purkinje cells (Jeste et al., 1984) and neuronal depletion in the dentate nucleus (Rodda, 1981).

At the ultrastructural level in HD, investigations have revealed deposits of cerebrosides and fatty acids, increased lipofuscin in neurones, dysfunction of the smooth endoplasmic reticulum and Golgi

vesicles and mitochondria, loss of presynaptic endings, axonal losses, dysfunction and lipofuscin accumulation in astrocytes (Tellez-Nagel et al., 1973; Goebel et al., 1978; Roisin et al., 1976). There is also evidence suggesting membrane changes in HD, including alterations to fibroblasts, lymphocyte capping and red blood cells (Butterfield and Markesbery, 1981; Pettegrew et al., 1987).

1.4 Transmitter neurochemistry

1.4.1 GABAergic neurones

γ-aminobutyric acid (GABA) is a major inhibitory neurotransmitter and is widely distributed thoughout the brain, together with its biosynthetic enzyme, glutamic acid decarboxylase (GAD). Perry et al. (1973) made the first observation of reduced GABA concentrations in the brain in HD. They reported losses in the putamen/pallidum (62% loss), caudate (40% loss), s.nigra (60% loss) and temporal and occipital cortex (32% loss), but with no significant changes in the frontal cortex or the cerebellum. Subsequent studies have confirmed these losses (Urquhart et al., 1975; Bird and Iversen, 1974; Spokes et al., 1979; Gramsbergen et al., 1986) and extended them to include the nucleus accumbens (47% loss), lateral pallidum (38% loss), subthalamic nucleus (19% loss), venterolateral thalamic nucleus (24% loss), s.nigra (25% loss) and frontal cortex (5% loss), although the hippocampus, amygdala, dentate nucleus, premotor cortex and cerebellum were described as showing no change (Spokes et al., 1980). More recently, Ellison et al. (1987) made an extensive study of reductions of GABA concentrations in the brain in HD, to search for an association with the grades of severity of the disease. They concluded that GABA deficits correlated positively

with the severity grade. They also identified some regional gradients of GABA deficits; thus, (caudate > putamen > nucleus accumbens) and (s.nigra pars reticulata > s.nigra pars compacta). However, they reported that cortical GABA concentrations were not significantly changed in HD, as were levels in the hippocampus, claustrum, subthalamic nucleus, venterolateral and dorsomedial thalamic nuclei. Of note was a 50% increase in GABA concentrations in the anterior thalamic nucleus. Kish et al.(1983) and Beal et al. (1988d) both found no significant deficit in GABA concentrations in the cerebellum in HD.

Changes in GAD activity have also been well documented in HD, but unfortunately some studies did not control adequately for agonal state of the patient, which has been shown to influence the activity of GAD (Spokes et al., 1979). Thus, only recent investigations are properly controlled. Spokes et al. (1979, 1980) described a 54% reduction of GAD activity in the putamen, as well as the caudate (50% loss) and the lateral pallidum (45% loss). All other areas examined showed no significant reductions (medial pallidum, s.nigra pars reticulata, s.nigra pars compacta, nucleus accumbens, cerebellum, dentate nucleus, red nucleus, olivary nucleus, motor cortex and hippocampus). However, Yates et al. (1990) reported a reduction in the frontal cortex. Activity of GABA transaminase (GABA-T), the metabolic enzyme for GABA, has been little studied in the HD brain. Urquhart et al. (1975) reported no change in the striatum, but with only two cases this is merely an observation. Carter (1984) showed a 40-50% reduction in the activity of GABA-T in the putamen in HD.

Lloyd et al. (1980) reviewed the literature and

challenged the early report of Enna et al.(1976a) that found no significant changes in GABA receptor binding in the brain in HD. Lloyd provided data suggesting that there was decreased GABA binding in the caudate (80% loss) and the putamen (50-75% loss), whilst cerebellar binding was 100% increased and the s.nigra and cortex remained not significantly changed (Lloyd et al., 1977; Olsen et al., 1979; Reisine et al., 1979; Iversen et al., 1979). Further reports described a reduction of binding in the putamen (55% loss), venterolateral thalamus but with no significant change in the cortex (Penney and Young, 1982; Young et al., 1988). Lloyd noted that although there was no significant change in the frontal cortex, this was due to increased affinity together with decreased Bmax. In addition, the Kd in the cerebellum in HD was three times lower than that in the controls. Furthermore, they suggested that there may be phospholipid regulation of GABA and the receptor, which could be abnormal in HD. Cross and Waddington (1981) described a difference in the s.nigra in HD, contrary to previous reports. They found 200% increase in the density of high-affinity sites and a small increase in the density of low-affinity sites. The benzodiazepine site is one subunit of a macromolecular complex consisting of a GABA A receptor recognition site, chloride ionophore, benzodiazepine site and barbiturate-picrotoxin receptor (Olsen, 1981; Rothstein et al., 1989). Benzodiazepines are thought to be positive allosteric modulators of GABA function and may act by displacing an endogenous inhibitor of GABA binding and potentiate GABA action (Rothstein et al., 1989). Several groups have measured benzodiazepine binding in the brain in HD (Reisine et al., 1978; Young et al., 1988; Kish et al., 1983). Reisine et al.(1978) found a 35% reduction

in the number of sites together with reduced affinity in the putamen in HD, which could be normalised by adding GABA. They also reported a 25% increase in binding in the frontal cortex and cerebellum, consistent with another study by Trifeletti et al. (1987), as well as an increase in the s.nigra (but with no change in affinity). Kish et al. (1983) produced contrary results showing no significant change in binding in the cerebellum, and Young et al. (1988) demonstrated a 55% loss of binding in the putamen, but no change in the cortex. Reisine et al suggested that GABA losses in the putamen may be responsible for the reduced affinity of benzodiazepine receptors, however this did not seem to occur in the cortex, where there are also GABA losses in HD. An associated parameter is diazepam-binding inhibitor (which may be the endogenous ligand for the benzodiazepine receptor). Ball et al.(1988) determined diazepam-binding inhibitor in the brain in HD, finding increases (50-90%) in the caudate, putamen, pallidum and nucleus accumbens, but no significant difference in the cortex, hippocampus or hypothalamus. GABA uptake sites (using binding of radio-labelled nipecotic acid) have identified an actual decrease of neurone terminals in HD in the caudate (52% loss) and putamen (67% loss), but not in the temporal cortex (Simpson et al., 1987). Here, the caudate deficit appears less than their 74% loss of GABA concentrations. However, Czudek and Reynolds (1990) reported 58% loss of uptake binding in the putamen, compared with 62% loss of GABA levels, thus showing no difference between the alternative measures in HD. Furthermore, they suggested that the methodology of Simpson et al. (1987) under-estimated the number of uptake sites, but unfortunately, the cases in the Czudek study are too small for statistical analysis.

Thus, the evidence appears to reflect the selective loss of GABAergic neurones (particularly striatonigral and intrinsic pathways) which is described in HD. Consequently there is decreased inhibition of the dopaminergic nigrostriatal pathway which has been suggested to be involved in the production of chorea. Furthermore, differential losses of the GABAergic striatopallidal pathways result in the alteration of the medial/lateral balance. There is also an association of GABA changes with affective disorder, particularly depression (Peyser and Folstein, 1990) which is a common feature of HD (see section 8.1).

1.4.2 Dopaminergic neurones

The nigrostriatal dopaminergic pathway was initially thought to be overactive in HD, but evidence does not support this theory. There have been many inconsistent reports regarding the concentrations of DA in the striatum in HD. Studies have been made reporting a significant increase in the putamen (Melamed et al., 1982), caudate (Bernheimer and Hornykiewicz, 1973), both areas (Spokes, 1980) or neither areas (Bird and Iversen, 1974; Reynolds and Garrett, 1986). Kish et al.(1987) described a gradient of losses of DA concentrations in both areas, reaching significance in the caudal caudate. The variability in these results has been suggested to reflect an increased range of DA concentrations in the striatum in HD (Reynolds and Garrett, 1986), or differences between the studies in the control of postmortem delay (Kish et al., 1987), or perhaps a drug effect (see section 3.4). Increased concentrations of DA have been reported in other nonstriatal regions including the lateral pallidum (by 71%), medial pallidum (by 79%), nucleus

accumbens (by 87%), s.nigra pars compacta (by 34%) whereas there are no reported significant differences in the s.nigra pars reticulata, red nucleus, anterior perforated substance or septal nucleus (Spokes, 1980). However, Kish et al.(1987) found no significant change in the nucleus accumbens or the s.nigra.

Concentrations of homovanillic acid (HVA, the major metabolite of DA) have been previously used to reflect the turnover of the DA system. In HD, concentrations of HVA appear to be greatly reduced in the caudate (48% loss) and putamen (50% loss) (Reynolds and Garrett, 1986), consistent with reports by Kish et al.(1987) and Walsh et al. (1982), whereas, Melamed et al.(1982) found no significant difference. A trend towards reduced HVA concentrations has also been demonstrated in the nucleus accumbens and s.nigra pars reticulata (Walsh et al., 1982; Kish et al., 1987).

The determination of tyrosine hydroxylase activity (the rate-limiting enzyme in the formation of DA) has demonstrated no changes in the caudate, putamen, nucleus accumbens, s.nigra, lateral and medial pallidum or hippocampus in HD (McGeer and McGeer, 1976b). However, Bird and Coyle (1986) made a similar study showing a 30% increase in the caudate and a 300% increase in the s.nigra.

A study of the activities of the enzymes monoamine oxidase A and B (MAO A,B) was made in HD; DA is metabolised mainly by MAO B in the human brain. Mann et al. (1986) found the kinetics of MAO A to be not significantly changed in the caudate and the frontal cortex. However, MAO B activity was increased in the frontal cortex (by 26%) and significantly in the caudate (by 260%).

Two types of DA receptors have been investigated in HD: D1 and D2. A deficit in the binding to D1 receptors has been reported to occur in the striatum (80-90% loss) with a relative sparing of the nucleus accumbens (50% loss) (Joyce et al., 1988; Cross and Rossor, 1983). A 70% loss has also been described in the s.nigra in HD, that correlates with an adenylate cyclase deficit (Filloux et al., 1990). Recently, DeKeyser et al. (1989a) identified two subtypes of D1 receptors : GTP sensitive (GS) and GTP insensitive (GI). In the brain in HD they demonstrated a 58% loss of binding in the putamen (50% increase of GI and 100% loss of GS), 20% loss in the pallidum and no significant change in the amygdala (100% loss of GS and 100% increase of GI) and the cortex (no changes). They suggested that there may be abnormal coupling of GS-D1 receptors with a G-protein in HD. In the brain in HD, D2 binding losses of 50% have been reported in the caudate and putamen (Seeman et al., 1987; Reisine et al., 1977; Cross and Rossor, 1983; Joyce et al., 1988). Here too, De Keyser et al. (1989b) subdivided D2 receptors into two categories: GS and GI. They demonstrated a 28% loss in the putamen (100% loss of GI and no loss of GS) and 100% loss in the pallidum (100% loss of GI). These findings were challenged by Reynolds et al. (1990b) who reported no significant change in D2 receptor binding in the lateral pallidum in HD, which contradicts the above report. A further study by Seeman et al. (1989) suggested that the link between D1 and D2 receptors was reduced in HD (and schizophrenia). D1-D2 interactions, perhaps mediated by a G-protein, were reported to be missing in over half the HD cases analysed. There is evidence from one study that DA uptake sites are increased by 65% in the putamen and caudate in HD (Oldroyd et al., 1990).

Thus, the overall evidence would seem to reflect an intact nigrostriatal pathway, but with down-regulation resulting in reduced activity. Therefore, the initial view of an overactive system in HD may only be relative, reflecting a lack of inhibition by GABA and acetylcholine. As glutamate affects DA release (Roberts and Andersen, 1979) and DA has been suggested possibly to affect corticostriatal glutamatergic transmission (Kornhuber and Kornhuber, 1986) the glutamate dysfunction in HD may be associated with dopaminergic changes. Furthermore, dopaminergic alterations in limbic regions in HD may have some connection with symptoms such as schizophreniform psychosis (Reynolds, 1983).

1.4.3 Cholinergic neurones

In the striatum the cholinergic system appears to be made up of interneurones. Acetylcholine itself shows strong postmortem losses, thus, markers for the system have often been measured. These include the activity of the biosynthetic enzyme, cholineacetyltransferase (CAT) and acetylcholinesterase (ACE). ACE is not always localised in cholinergic neurones, therefore changes of its activity may not identify cholinergic deficits. Slight increases or no significant changes were observed of ACE activity in HD in both cortical and subcortical regions (Hammond and Brimijoin, 1988; McGeer and McGeer, 1976b; Stahl and Swanson, 1974). Many studies have investigated CAT activity in the brain in HD. Bird and Iversen (1974) described 'patchy' losses in HD in the caudate (50% loss) and putamen (50% loss) and subdivided the groups to select a 'normal' activity group, as compared with 85% losses in the remainder.

No significant changes were found in the frontal cortex. The results of McGeer and McGeer (1976b), Spokes (1980) and Cross (1986b) confirm these findings as well as identifying deficits in the locus coeruleus, hippocampus and septal nucleus in HD. Neither group found a significant deficit in the nucleus accumbens (and Spokes reports a slight increase). The pallidum (lateral and medial), cerebellum, cortex, s.nigra (pars compacta and pars reticulata) and red nucleus were all not significantly changed. Aquilonius et al. (1975) made an investigation of the subdivided striatum in HD (but only using two brains). They reported a patchy gradient of deficits across the neostriatum with a maximum loss of 90% in the paraventricular caudate and rostral caudate. Other nonstriatal regions were found to have no significant changes, except for an increase in the cingulate gyrus.

Muscarinic cholinergic receptors have been investigated in binding studies in the brain in HD. Enna et al.(1976a) identified a 50% loss of binding in the putamen and the pallidum. This confirmed the findings of Hiley and Bird (1974) and Young et al.(1988), although no significant changes were reported in the cortex. Wastek et al.(1976, 1978) extended the negative findings to include 24 other brain regions.

These changes suggest that there appears to be deficits in the cholinergic system, resulting in reduced antagonism of the dopaminergic nigrostriatal pathway. A possible cholinergic association with dementia may be evident in HD, as cortical deficits are reported to occur with dementia in PD and AD (Perry et al., 1983; Rossor et al., 1984).

1.4.4 Noradrenergic neurones

A pathway of noradrenergic neurones originates from the locus coeruleus, but as there are only small concentrations of NA in the basal ganglia, it has been little studied in HD. One investigation (Spokes, 1980) identified increases in NA concentrations in HD in the caudate (by 63%), lateral pallidum (by 38%), nucleus accumbens (by 46%) and s.nigra pars reticulata (by 40%). The putamen, medial pallidum, s.nigra compacta, red nucleus, septal nucleus and anterior perforated substance showed no significant changes. 3-Methoxy-4-hydroxyphenylethylene glycol, a metabolite of NA, was not significantly altered in the CSF of HD patients (Chase, 1973). Enna et al. (1976a,b) described results indicating that there was no significant difference in the density of B-adrenergic receptors in the caudate or the putamen in HD, but there was a deficit in the pallidum.

It is possible that NA and DA may act synergistically (Spokes, 1980), therefore, any changes may influence the nigrostriatal DA system. The effects of NA in other brain regions (eg. cortex) are more likely to be linked with psychiatric symptoms or dementia or affective disorder (Peyser and Folstein, 1990), than with dyskinesia.

1.4.5 Serotonergic neurones

A major striatal serotonergic pathway derives from the raphe nucleus. There are only a few studies reporting the serotonin (5HT) system in the brain in HD. Bernheimer and Hornykiewicz (1973) described normal concentrations of 5HT in the brain in HD, with a slight increase in the putamen, but only

reported from four cases. Recently, Kish et al.(1987) made a study of 5HT concentrations throughout the striatum, nucleus accumbens and s.nigra (rostral) in HD. They identified a general increase throughout the HD'striatum, reaching significance in the rostral caudate and intermediate putamen, but with no significant change in the nucleus accumbens or the s.nigra. Furthermore, they measured concentrations of the major metabolite of 5HT, 5-hydroxyindoleacetic acid (5HIAA), and found a similar pattern (except for the head of the caudate which was not significantly altered).

Binding studies provide evidence that suggests that 5HT2 receptors are not significantly altered in the HD brain in the putamen, frontal cortex and temporal cortex (Cross et al., 1986b). However, Cross et al.(1986b) also reported deficits of 5HT1 receptors in the putamen (50% loss) and hippocampus (36% loss) but not significantly altered in the frontal or temporal cortex in HD. These findings were consistent with the results of Enna et al. (1976b) and Waeber and Palacios (1989) that extended the deficit to include the caudate, nucleus accumbens, pallidum (medial and lateral) and s.nigra (pars compacta and pars reticulata), but found no significant effect in the hippocampus or the cortex. 5HT uptake sites showed a 64% increase in the putamen, but no significant change in the caudate in the brain in HD (Cross et al., 1986c).

5HT has a possible effect in the production of myoclonus and hyperactivity and chorea (Gerson and Baldessarini, 1980; Ringel et al., 1973). Decreased 5HT activity has also been associated with depression, aggression and dementia (Peyser and Folstein, 1990; Bowen et al., 1983). Tryptophan concentrations have also been measured in the brain

in HD: Perry et al. (1973) found no significant change, as did Beal et al. (1990). Total tryptophan in the plasma in HD was not significantly altered, whereas, free tryptophan was abnormally reduced after fasting or hypoglycaemia (Phillipson and Bird, 1977).

1.4.6 Glutamatergic neurones

A major input into the striatum is the excitatory corticostriatal pathway, which is known to be glutamatergic (Fonnum et al., 1981). There is also a glutamatergic projection from the subthalamic nucleus to the medial and lateral pallidum (Robertson et al., 1989). However, glutamate is also an important intermediate of metabolism and thus altered concentrations do not necessarily reflect neuronal losses . Diminished concentrations of glutamate in the brain in HD have been described in the caudate and the putamen (30% loss), but frontal cortex, cerebellum and s.nigra remained not significantly changed (Perry et al., 1973, 1982; Gramsbergen et al., 1986; Beal et al., 1988d). Ellison et al. (1987) made a comprehensive investigation of the regional distribution of glutamate concentrations in the brain in HD. They found deficits in severe grades of the disease, in the caudate (39% loss), putamen (27% loss), nucleus accumbens (19% loss) and in frontal (BA 9), premotor (BA 6), postcentral (BA 3-1-2) and occipital (BA 17) cortex (16-20% losses). The s.nigra pars compacta showed an increase, but all other regions measured showed no significant changes (s.nigra pars reticulata, medial and lateral pallidum, hippocampus, claustrum, subthalamic nucleus, thalamus and other cortical regions). Cortical losses in HD are also consistent with Kremzner et al. (1979).

A possible marker for the glutamatergic system is the enzyme, ornithine aminotransferase, which has been suggested to be concentrated in glutamatergic terminals (Wong et al., 1982). They demonstrated reduced activity of ornithine aminotransferase in the caudate, putamen, parietal and frontal cortex (34-49% losses) in HD.

High-affinity uptake sites were also reported (Cross et al., 1986c) to be diminished in the caudate (70% loss), putamen (60% loss) and hippocampus (30% loss), whereas the temporal cortex remained not significantly changed in HD. Greenamyre et al. (1985) and Young et al. (1988) measured glutamate receptor binding sites in the HD brain. They identified a loss of total glutamate and quisqualate-sensitive binding in the caudate and putamen (67% loss), whereas there was no significant change in the cortex, claustrum and basal nucleus. However, in the putamen, N-methyl-D-aspartate (NMDA)-sensitive binding showed a 93% deficit, although in the cortex (insular), there was no significant reduction. The phencyclidine receptor (associated with the NMDA receptor complex) showed 67% loss in the putamen in HD, with no significant change in the cortex. Beaumont et al. (1979) determined the density of kainate binding in HD brain. They reported a deficit in the caudate (55% loss) and putamen (53% loss), with no significant change in the pallidum, frontal cortex or cerebellum.

These changes reflect the loss of the corticostriatal pathway and cell losses from the striatum (matrix), which receives the major cortical input (Seto-Ohshima et al., 1988a). Other evidence supporting the theory of glutamatergic losses, is

the atrophy of layer 5 of the cortex, the origin of the corticostriatal fibres (Bruyn et al., 1979). Lesions of the subthalamic-pallidal pathway has been shown to be important in the balance between medial and lateral pallidum (Crossman et al., 1988). Thus, if there is a deficit, there may be dyskinetic consequences (see section 4.1). The changes in the glutamatergic system are potentially of relevance to the 'excitotoxic hypothesis' regarding the aetiology of the disease, which is thought to be mediated by glutamate receptors (especially NMDA) (Bruyn and Stoof, 1990). Furthermore, cortical losses of amino acids have been demonstrated to occur in other dementing disorders, suggesting a common feature (Rossor et al., 1984; Reynolds and Warner, 1988). Glutamate and DA may also modulate the release of each other (as discussed in section 3.2) (Roberts and Andersen, 1979; Kornhuber and Kornhuber, 1986).

1.4.6 Other amino acids

Aspartate is also a possible neurotransmitter in the corticostriatal tract and in cerebellar climbing fibres (Fonnum, 1984; Wiklund et al., 1982). Ellison et al. (1987) and Perry et al. (1973) found no significant difference in aspartate concentrations in the HD brain, but Beal et al. (1988d) found a 21% increase in the cerebellum and Gramsbergen et al. (1986) reported a slight increase in the s.nigra. The meaning of such increases is unclear, but it may reflect increased aspartate system or a selective sparing in a region of atrophy.

Taurine is also a putative neurotransmitter (Baskin et al., 1976) which is derived from cysteine metabolism (Perry et al., 1975). Gramsbergen et al.(1986) reported a slight increase in taurine

concentrations in the putamen in HD, but Ellison et al. (1987) and Perry et al. (1973) found no significant changes. Beal et al. (1990a) reported increased cysteine concentrations in the putamen in HD.

Glycine is thought to have a major role at the NMDA receptor complex, potentiating responses (Foster and Kemp, 1989). It also has an inhibitory role in the central nervous system. Thus any changes in HD may have relevance to the 'excitotoxic hypothesis' of the disease (see section 6.3). Perry et al. (1973) reported no significant changes in glycine throughout the brain in HD.

1.5 Neuropeptides and enzymes

1.5.1 Neuropeptides

Many of the neuropeptides have been suggested to be neuromodulatory to, as well as reacting to dopaminergic changes. Changes in immunocytochemical activity or concentrations have been well established in HD, thus identifying losses of subpopulations of neurones. Substance P is known to be present in a subpopulation of medium spiny GABAergic neurones projecting from the striatum to the medial pallidum and s.nigra (pars compacta and pars reticulata) (Reiner et al., 1988). Extensive losses of 80-90% of substance P have been described in the lateral and medial pallidum, s.nigra (pars compacta and pars reticulata) and caudate and putamen (Emson, 1986; Arai et al., 1987; Gale et al., 1978; Kanazawa et al., 1977; Waters et al., 1988), but no significant changes in the amygdala, hippocampus or frontal and temporal cortex. Beal et al. (1988a) attempted to correlate substance P deficits with gradings of severity of pathology.

They found substantial losses of substance P throughout the basal ganglia (including the nucleus accumbens), that strongly correlated with striatal neuronal losses. Furthermore, they described losses in non-striatal regions including the subthalamic nucleus and stria terminalis, but no significant changes in the thalamus, claustrum, hippocampus, locus coeruleus, lateral geniculate and red nucleus. Of 24 cortical regions studied, only premotor, frontal eye field and frontal areas (BA 6,8,9) showed 20-30% increase. The relevance of these changes must be understood in terms of co-localisation described above. These GABAergic spiny neurones are lost in HD, thus pallidal, nigral and striatal regions show deficits of substance P. The deficit in the subthalamic nucleus was described by Beal et al. (1988a) as reflecting a possible loss of substance P-containing pallidal-subthalamic afferents, but this is only speculative. Substance P has been reported to co-localise with NPY or SS in some cortical neurones, thus cortical increases would identify selective sparing (Jones and Hendry, 1986). The role of substance P is thought to be excitatory and modulatory for DA (Beal et al., 1988a). Related to substance P, are neurokinin A, neurokinin B and neuropeptide K (all of which derive from the same two precursors). In HD, neurokinin A showed losses in all areas except for the cortex; neurokinin B was reduced in the s.nigra and medial pallidum and neuropeptide K was reduced in the s.nigra and frontal cortex (Arai et al., 1987). The normal function of these peptides and their possible relevance to HD is unknown.

Somatostatin (SS) has been described to be increased in HD in the caudate (by 100%) and nucleus accumbens (by 300%), with no significant change in the amygdala, hypothalamus or cortex (Nemeroff et al.,

1983). Increases were also reported in the putamen and pallidum (medial and lateral), but no significant changes in the s.nigra (pars compacta and pars reticulata), hippocampus or hypothalamus (Emson, 1986; Aronin et al., 1983). SS-related peptides were also reported to be increased (by 55%) in the putamen in HD (Sperk et al., 1987). Beal et al. (1988c) again tried to correlate changes with severity of striatal neuronal degeneration. They confirmed the increases (by 300%) of SS in the basal ganglia (except the medial pallidum) and extended the regions with increases to include the red nucleus, locus coeruleus and several cortical areas. No association was evident with pathological grading of severity. There is strong evidence that SS co-localises with NPY and NADPH-d in striatal and in cortical (GABAergic) medium aspiny neurones (Ferrante et al., 1987b). Studies of NPY in the brain in HD reveal similar patterns of increases (by 250%) throughout the basal ganglia (except the medial pallidum) (Emson, 1986; Beal et al., 1988c) and also in the subthalamic nucleus, s.nigra pars compacta, claustrum, thalamus, stria terminalis and locus coeruleus. Increases were also found in many regions of the cortex. Here too, there was no correlation with pathological severity. The pattern of increase for SS and NPY, reflects the selective sparing of this subpopulation of aspiny medium neurones in HD; thus, within the cortex identifies GABAergic neurones that are not lost. It was noted that the medial pallidum showed no significant increases in SS or NPY (Dawbarn et al., 1985). Kish et al. (1987) made a suggestion that 'preservation of 5HT2 receptor-containing neurones corresponds with sparing of SS-containing neurones in HD', but there is no strong evidence for this. SS is thought to be associated with increased production and release of DA (Aronin et al., 1983).

Met-enkephalin has been measured in HD brain by Emson (1986) who reported losses (50%) in the pallidum (lateral and medial) and s.nigra (pars compacta and pars reticulata), but no significant changes in the caudate or putamen. Met-enkephalin co-localises with the GABAergic striatal projection to the lateral pallidum, which is lost in HD (Reiner et al., 1988). Thus, a met-enkephalin deficit would be expected. Met-enkephalin may modulate DA turnover (Diamond and Borison, 1978). A further opioid peptide is dynorphin, which is lost from the pallidum and s.nigra and striatum in HD (Waters et al., 1988; Seizinger et al., 1986; Dawbarn et al., 1986).

The neuropeptides, cholecystokinin (CCK) and vasoactive intestinal peptide (VIP) were also determined in the brain in HD (Emson, 1986). VIP was described as not significantly changed throughout the brain, whereas CCK showed losses (50%) in the pallidum and the s.nigra, but was not significantly altered in the striatum. Furthermore, there was reduced CCK binding in the basal ganglia (70% loss) and the cortex (43% loss) (Hays et al., 1981). CCK defines a subpopulation of GABAergic cortical neurones, which appear to be lost in HD; whereas subcortically, CCK is thought to co-localise with DA in the ventero-tegmental and nucleus accumbens neurones, having an antagonistic effect (Hokfelt et al., 1980).

Nemeroff et al. (1983) and Emson (1986) measured neurotensin in the HD brain, finding a 50% increase in the caudate and medial pallidum, but no significant change in the nucleus accumbens, amygdala, hypothalamus, putamen, lateral pallidum or s.nigra. Neurotensin is also thought to co-localise

with DA (Hokfelt et al., 1984).

1.5.2 Peptides of the hypothalamus

Thyrotropin-releasing hormone (TRH) showed increases in the caudate and amygdala and putamen (300%) in HD, but no significant change in the hippocampus or the nucleus accumbens (Nemeroff et al., 1983; Emson, 1986; Spindel et al., 1980). A substantial increase (by 400%) of gonadotropin-releasing factor in the median eminence in HD was described by Bird et al. (1976). Angiotensin-converting enzyme (ACEZ) has been reported to be decreased in the caudate, putamen, pallidum, s.nigra and nucleus accumbens, but not significantly altered in the cortex in HD (Arregui et al., 1978; Butterworth, 1986). Angiotensin is reportedly neuroactive and ACEZ is described to be localised in the striatonigral pathway (Arregui et al., 1978). One HD case report has been published describing 'dramatic deterioration of clinical state' whilst taking ACEZ inhibitor, with vast improvement on withdrawal of the drug (Goldblatt and Bryer, 1987).

1.5.3 Pituitary hormones

DA has been shown to inhibit prolactin secretion, whereas GABA increases it (Hayden, 1981). Hayden reviewed reports of prolactin in HD: 4 studies suggested that basal levels were not significantly changed, two reported an increase and two a decrease. However, responses to various stimuli (TRH, L-Dopa, bromocryptine) suggested an impaired system. Hayden also reviewed the literature on growth hormone regulation in HD. Growth hormone release is thought to be stimulated by DA. There is a general consensus that basal levels of growth hormone are normal in HD (in 6/7 studies). Stimuli

(eg. insulin, L-Dopa, bromocryptine) appeared to have increased growth hormone responses in HD patients.

1 5.4 Enzyme changes in HD

Butterworth (1986) made an extensive study of many enzyme markers for neurones, glia and endothelial cells in the caudate in HD. There were losses of all neuronal marker enzymes (ACEZ, thermolysin-like metalloendopeptidase, alanine aminopeptidase, glutamate-oxaloacetate transaminase). Glial marker enzymes showed varying changes (glutamine synthetase was reduced, ß-glucuronidase was increased and pyruvate carboxylase was variable). Glutamine synthetase was reported to be reduced in the putamen, cortex (frontal and temporal) and cerebellum (Carter, 1981). The endothelial enzymes (alkaline phosphatase and plasminogen activator) were not significantly changed in HD. Butterworth et al. (1985) also measured the activity of 4 other enzymes throughout the HD brain. Phosphate-activated glutaminase, succinic dehydrogenase and pyruvate dehydrogenase were reduced in the caudate, however, γ-glutamyl transpeptidase was increased. This is consistent with other reports (Zhu et al., 1990; Cross et al., 1986a), which suggested an increased γ-glutamyl transpeptidase activity may be a glial marker. Cross et al. (1986a) determined activities of many enzymes in the putamen in HD, using markers of subcellular organelles and acid hydrolases. A plasma membrane associated enzyme (5'nucleotidase) was not significantly changed, whereas γ-glutamyltransferase (described above) was increased (but not in the cortex and cerebellum). 2',3'-cyclic nucleotide phosphohydrolase, a myelin marker, was increased which is consistent with the demonstration of increased cholesterol and

sphingomyelin (Borri et al., 1967) reflecting relative axonal sparing. DNA concentrations were increased, as was one of the lysosomal markers (β-glucuronidase) however, the other one (acid phosphatase) was not significantly altered. Catalase (a peroxisome marker) and fumarase (a marker for mitochondria) were not significantly changed, but neutral α-glucosidase (a marker for endoplasmic reticulum) was reduced (but not significantly altered in the cortex and cerebellum). Glycoside hydrolases were variable: N-acetyl-ß-glucosaminidase, ß-glucosidase and α-fucosidase were all reduced, whereas α-galactosidase was increased. All the other enzymes in this group (ß-xylosidase, α-mannosidase, ß-galactosidase) were not significantly changed.

Cross concluded that ß-glucuronidase activity was a marker for neuronal degeneration and that α-glucosidase differences may reflect dysfunction of glycoconjugate processing, which may explain the specific degeneration of neurones in HD. Yates et al. (1990) reported reduced activity of pyruvate dehydrogenase in the caudate in HD and suggested that this also was a measure of neuronal losses. Furthermore, they stressed the importance of pH and lactate in the interpretation of neurochemical measurements, highlighting the need for controls of similar agonal state (see section 1.4). Bird and Coyle (1986) described further determinations of the enzymes involved in glucose metabolism (Bird et al., 1977). They reported specific losses, in the putamen and caudate, of phosphofructokinase and ouabain-insensitive ATPase, whereas in the pallidum, glutamate dehydrogenase was increased. There were no significant changes of hexokinase, glucose-6-phosphate dehydrogenase or lactic acid dehydrogenase; the cortex, hippocampus and cerebellum showed no significant changes. A further

enzyme (NADH: ubiquinone oxidoreductase (complex 1) measured in the platelets from patients with HD, was shown to be reduced. Other measures of mitochondrial function (succinate: cytochrome c oxidoreductase and cytochrome oxidase) were not significantly altered (Parker et al., 1990). They suggested that HD could be a genetic mutation of the nuclear coded subunits for this enzmye. A similar deficit has been noticed in PD, suggesting a common complex I defect (Shapira et al., 1989). Furthermore, Shapira (Mann et al., 1990) measured mitochondrial function in the putamen, caudate and cortex in HD finding decreases in complexes II and III, but not in I or IV. Thus, their hypothesis contrasted HD with PD (where only complex I is abnormal).

1.5.5 Calcium-associated proteins

Calbindin-containing neurones are localised in the striatal matrix, in medium spiny neurones, thus there are losses of these neurones in HD (Seto-Oshima et al., 1988b). Calcineurin has been described as providing a marker for striatal efferents, as it is found only in the striatum and striatal projections (Goto et al., 1989). They reported striatal losses and also in the pallidum (the dorsal part being less affected) in HD (but only one case). They measured synaptophysin in the same brain, as a vesicular marker for pallidal afferents. Synaptophysin also shows losses in the pallidum (also with the dorsal part less affected). These losses were not as great as the calcineurin deficit and were not marking the same population of neurones.

1.6 Neuropharmacology

At present there is no cure and no effective treatment to stop the disease progressing. However, individual symptoms can be addressed with some success. Hayden (1981) reviewed some aspects of drug therapy in HD.

The most common drugs used in HD are those which interact with the DA system . In the UK, tetrabenazine is the drug of choice (Jankovic, 1982) for the alleviation of chorea, although its use is banned in other countries. Like reserpine it depletes monoamine storage and thus reduces DA activity. However, it also acts on other monoamines, such as 5HT and NA, which are more likely to mediate the serious side effects associated with its use (drowsiness, depression and suicide) (Lane et al., 1976). Neuroleptics are also used; they act as postsynaptic DA antagonists. These drugs include butyrophenones such as haloperidol and phenothiazines eg. perphenazine, the USA drug of choice for reducing chorea (Fahn, 1979). Common side effects of DA antagonists include Parkinsonism, akathesia, dystonia and tardive dyskinesia which may limit their use. L-Dopa predictively aggravates chorea, but some DA agonists (eg. apomorphine, bromocryptine) at low doses unexpectedly reduced choreiform movements. At higher doses, the chorea was worsened and these actions were postulated to be mediated via presynaptic dopaminergic autoreceptors (Frattiola et al., 1977; Carlsson, 1976). Substituted benzamides (e.g. sulpiride) were described to be more specific with less side effects (Zanglein et al., 1978). Combination therapy of a D2 receptor blocker and a DA storage blocker has been described as a better therapy for the alleviation of chorea. This was

longer lasting (after an initial deterioration period) and free from the usual psychiatric side effects (Fog and Pakkenberg, 1970). The psychosis of HD responds to neuroleptics, but with higher doses than are needed to reduce chorea (Hayden, 1981). Clozapine has also been used to treat HD (Dose, 1991; Sajatovic et al., 1991) with limited success in some patients that had problems associated with other traditional neuroleptics.

One obvious form of therapy in HD appeared to be the restoration of GABA levels, by replacement therapy or by reducing its breakdown. However, the drugs which influence the GABA system seem in general not to be effective in the reduction of chorea, and side effects are possible from the nonspecific effects of GABA in other regions of the brain. Receptor agonists have been tried (muscimol, baclofen) (Shoulson et al., 1978, 1989) as have GABA-T inhibitors (isoniazid, aminooxyacetate) (McLean, 1982; Perry et al., 1980), none of which have proven consistently effective in the amelioration of chorea. Associated with GABAergic transmission, benzodiazepines have been used in the treatment of HD, usually as sedatives, and reports suggest some success in the reduction of chorea (Peiris et al., 1976). However, tolerance may occur which limits their use.

A further obvious line of therapy has been influencing the cholinergic system. This can be achieved in several ways; by providing choline (Aquilonius and Eckernas, 1977) or deanol, which is converted to acetylcholine (Reibling et al., 1975), or by using anticholinesterases (physostigmine) (Tarsy et al., 1974). None of these approaches were successful in the reduction of chorea, however, anticholinergics (benzotropine) have been reported

to increase movements (Klawans and Rubovits, 1972).

Drugs affecting the noradrenergic system have also been used in HD, but with little effect. Antagonists (thymoxamine and propanolol) were tried, with the latter ameliorating chorea slightly and also used in the treatment of aggression in HD (Nutt et al., 1979).

Influencing the serotonergic system is a further approach to pharmacological therapy, but findings have been inconsistent. Tryptophan administration has been reported to have no effect on chorea (McLeod and Horne, 1972), whereas 5-hydroxytryptophan has been described as increasing choreiform movement (Lee, 1968). However, 5HT antagonists (methysergide) and agents which reduce 5HT synthesis (parachlorophenylalanine) both have no ameliorative effects on chorea (Ringel et al., 1973).

Tricyclics and lithium have also been used with some success in the treatment of psychiatric symptoms of the disease, but not with the chorea (Vestergaard et al., 1977; Chiu, 1979). Corticosteroids have also had no consistent success (Brown et al., 1979). A drug that depletes SS levels (cysteamine) was tried in one study, but with no beneficial results (Shults et al., 1986). Another observation comes from a single case report, suggesting that that clinical symptoms of HD were aggravated by treatment with ACEZ inhibitor, and improved on withdrawal of the drug (see section 1.5) (Arregui et al., 1978).

1.7 Imaging techniques

Imaging techniques can provide a useful tool in the study of HD. Structural and metabolic changes can be investigated, although specific diagnosis cannot be made solely employing these measures. There have been many attempts to correlate early changes that may predict the disease; however, clinically, they are used as part of a battery of testing of presymptomatic patients, rather than as individual tests. The results of such studies have been inconclusive, especially as the longitudinal evidence will take many years to collect.

1.7.1 Computed tomography (CT) imaging

CT measures of striatal atrophy in HD (e.g. bicaudate ratio) have been reported that correlated with the progression of the disease symptoms, although it has been suggested that cortical cell losses would need more sensitive methods for accurate assessment (Bamford et al., 1989). Cognitive and oculomotor impairment was suggested to be associated with measures of caudate atrophy in this and other studies (Bamford et al., 1989; Starkstein et al., 1988, 1989), but not with cortical changes (Starkstein et al., 1988,1989). They determined both sub-cortical measures (bicaudate ratio, bifrontal ratio and third ventricular ratio) and cortical measures (frontal fissure ratio and cortical sulci ratio) in HD. Sub-cortical measures were associated with duration of disease, whereas cortical measures were linked with age, however, these measures were independent of each other. The bicaudate ratio was described as a useful diagnostic parameter (even in early HD).

1.7.2 Magnetic resonance imaging (MRI)

Lebrun-Grandie et al. (1988) reported focal atrophy in caudate that was less pronounced in the putamen, and associated with diffuse cortical atrophy. Reid et al. (1988) and Simmons et al. (1986) also reported similar changes using MRI to study HD.

1.7.3 Single photon emission computed tomography (SPECT) imaging

Reid et al. (1988) made a study in HD using SPECT and identified a reduction in blood flow in the caudate in HD. This was confirmed by comparison with MRI (and neuropathological findings, in one case).

1.7.4 Positron emission tomography (PET) imaging

Many PET studies have been made in HD (using mainly 18 fluorodeoxyglucose, FDG) (Young et al., 1986; Kuhl et al., 1984; Mazziota et al., 1987; Weinberger et al., 1988; Kuwert et al., 1990). Hypometabolism in the caudate and putamen was common to all studies, however, cortical hypometabolism was only described by Kuwert et al. (1989, 1990). Changes in some cortical regions were related to cognitive symptoms, but caudate measures showed a stronger correlation (Kuwert et al., 1990; Weinberger et al., 1988). Further associations were described by Young et al.(1986): putamen indices with motor function; thalamic metabolism with dystonia and caudate with bradykinesia and functional capacity.

1.7.5 Presymptomatic studies

People at risk to HD have been examined in many studies to try to get early diagnosis, or prediction of the disease, using four main approaches: neurological, metabolic, structural and genetic (all studied concurrently by Grafton et al., 1990). Many PET or CT investigations have been made, comparing changes with those found in HD itself. PET was suggested to reveal much earlier changes (especially in the caudate), as compared to CT (Kuhl et al., 1984; Mazziota et al., 1987) and thus, was a more useful tool in the prediction of HD. The study of Mazziota provided evidence that reduced caudate glucose metabolism was obvious in the statistically-predicted number of at risk subjects, although genetic linkage and clinical follow up was imperative to prove the hypothesis. Grafton et al. (1990) produced evidence that altered PET measures precede clinical or structural abnormalities, using DNA marker-tested at risk subjects. This effect was especially obvious in the putamen, caudate, pallidum and precentral gyrus. Young et al. (1986) previously produced an investigation showing that 'soft neurological signs'could be used to predict HD, but the study of Grafton did not concur with these findings. 'Soft signs' include saccadic eye movement, optokinetic movement, co-ordination of finger to nose, finger tapping, rapid alternating of movements.

1.8 Aims of this project

The main aims of this project were to study the neurochemistry of HD using a large series of post mortem brain tissue taken from patients with HD and from matched controls with no previous history of neuropsychiatric disorder. There were two main

aims: firstly, to identify and characterise any altered parameters of neurotransmitter systems, especially in relation to the symptomatology of the disease. Secondly, to understand the role of neurotoxins in the aetiology of the disease, particularly endogenous compounds that may have aberrant mechanisms (see section 6.3).

Chapter 2

Materials and methods

2.1 General details regarding cases studied

Brain tissue, provided by the Cambridge Brain Bank, was prepared and stored as described previously (Spokes, 1979). This was obtained post mortem from patients neuropathologically confirmed as having Huntington's disease (HD) and from controls with no previous history of neuropsychiatric disease. These groups (Table 2.1) were matched for age and post mortem delay, however the control group contained fewer females, reflecting the low death rate of females of this age range. Furthermore the cause of death in HD is usually bronchopneumonia, whereas there are more acute deaths in the control group, thus agonal state is another potentially artefactual factor that must be considered.

The HD cases were clinically assessed retrospectively by a neurologist (Dr. Kenneth Heathfield) in order to determine the symptomatology. This 'global impression' was based on all information in the medical and nursing case notes. Thus drug history, age at onset and duration of the disease, the degree of chorea (mild, moderate and severe), and of dementia (absence, mild, moderate and severe), or the presence or absence of schizophreniform, depressive, aggressive and rigid features were assessed. Consequently it was possible to investigate any neurochemical correlates of these features, by sub-dividing from the major group.

Table 2.1

Details of subjects providing brain tissue post mortem

Values are means. Ranges in parentheses.

	Age (Years)	Sex	Post mortem (Hours)	Duration (Years)
Controls	57 (21-82)	24M 3F	31 (3-79)	-
Huntington's disease	56 (30-83)	23M 23F	43 (3-153)	12 (3-24)

Neurochemical measurement was carried out using specific regions of the brain. The areas of particular interest were the basal ganglia (caudate, putamen, medial and lateral pallidum), the substantia nigra (s.nigra) pars compacta, as well as limbic (amygdala and hippocampus) and cortical areas (frontal and temporal).

2.2 Limitations of human post mortem brain studies of neurotransmitters

It is often difficult to acquire post mortem brain tissue, especially in the case of rare disorders. Thus the numbers of cases in a series may well be limited, making statistical analysis inappropriate.

Post mortem delay from time of death until the freezing of the tissue is highly variable (e.g. ranging from 3-153 hours). Many neurochemical changes occur in the initial few hours following death, but are then stable in the long-term (e.g. increased ɣ-aminobutyric acid (GABA) concentrations). Thus although absolute values may not be accurate, they have been shown to correlate strongly with pre-mortem values.

Age, sex and agonal state inevitably vary and differences may have effects on specific compounds (e.g glutamic acid decarboxylase (GAD) activity is strongly affected by agonal state).

Differences associated with prior drug treatment provide a further variable that is unavoidable, particularly when dealing with neuropsychiatric disorders.

For all the above limitations, there is no ideal solution, but a compromise must be reached. Of

primary importance is the use of 'matched' controls which are treated in an identical fashion to the samples involved in the investigation. Thus any inherent methodological errors will be negated. If it is impossible to match individuals completely, then a statistically similar mean and range of values from group to group would be acceptable. However, parameters such as drug treatment will never be matched in these studies, so appropriate statistical tests must be used to rule out the effects of such factors on any reported changes.

2.3 Measurement of concentrations of monoamines and their metabolites

Concentrations of noradrenaline (NA), dopamine (DA) and its major metabolite homovanillic acid (HVA) and 5-hydroxytryptamine (5HT) and its metabolite 5-hydroxyindoleacetic acid (5HIAA) were measured using an adaptation of an established high-performance liquid chromatography (HPLC) technique (Reynolds, 1983). For most brain areas a simple 'direct' method (which did not use any extraction techniques) was used, but for hippocampus, amygdala and cortex, an extraction process was necessary in order to determine the very much smaller concentrations of NA and DA in these regions. These methods allowed the concurrent measurement of amino acids from the same homogenate, on a different HPLC system.

2.3.1 'Direct' method

Tissue (approx. 50 mg) was homogenised in 0.5 ml 0.1 M perchloric acid (PCA) using a glass/glass homogeniser. All solutions contained 0.1 mM ascorbate (an anti-oxidant) and 0.1 mM EDTA (a chelating agent). The homogenate was centrifuged at 12000 g for 3 minutes and the supernatant was

diluted if necessary with 0.1 M hydrochloric acid (HCl). Stock solutions (2 mg/ml in 0.1 M HCl) of standards were diluted to 50 ng/ml with 0.1 M HCl. 20 µl were injected onto the HPLC system, whilst 100 µl was diluted 1+4 vols 0.1 M disodiumtetraborate buffer (pH 9.5) and stored at -20°C for later analysis of amino acids.

2.3.2 Extraction method

An extraction process is necessary to concentrate the small amounts of DA and NA in the hippocampus, amygdala and especially the cortex. The other compounds (5HT, HVA and 5HIAA) can be determined using the same 'direct' method as described above. A large volume of supernatant is added to the alumina at high pH and under these conditions the catecholamines such as DA, NA and dihydroxybenzylamine (DHBA) adhere to the alumina, whereas other compounds are washed away. Rapidly decreasing the pH results in the elution of the catecholamines into a small volume; also there is improved chromatography due to the removal of many contaminants.

This method was used for the hippocampus and amygdala (for the cortex the concentrations of the standards and solutions were 10 fold smaller). Tissue (approx. 150 mg) was homogenised in 1.0 ml 0.1 M PCA containing 10 ng/ml DHBA as an internal standard, which was demonstrated not to co-elute with any sample peaks. The homogenate was centrifuged at 12000 g for 3 minutes and aliquots were injected, or diluted and frozen as described previously. The remaining supernatant (approx. 700 µl) was added to 25 mg alumina and 0.5 ml Tris (hydroxymethyl) methylamine (TRIS) buffer (0.1 M pH 8.6) for extraction at 4°C. The tube was gently

shaken for 10 minutes then allowed to settle. The supernatant was sucked off and washed rapidly with two successive 1 ml water washes. The remaining liquid was removed and 100 µl 0.4 M PCA was added to the alumina and gently agitated for 10 minutes, to elute the catecholamines. The eluate was removed and 50 µl were injected into the HPLC system. Standards solutions containing equivalent amounts (e.g. 10 ng) of NA, DA and DHBA were extracted onto the alumina in the same way.

2.3.3 HPLC characteristics

This isocratic reverse phase system used a Spherisorb ODS-2 5 µm (25 cm x 4.6 mm) column at 40°C, together with a 0.1 M phosphate/acetate buffer at pH 3.6 containing 2.5 mM octylsulphonate, 0.05 mM EDTA and 15% methanol. Quantification was achieved using an electrochemical (ED) detector (BAS) containing a glassy carbon electrode set at +0.75 V (vs. Ag/AgCl).

The retention time of peaks in samples were compared to the co-elution of authentic standards, and their identity further confirmed by the recovery of added standards to samples. Thus NA, DHBA, 5HIAA, DA, HVA and 5HT all produced single peaks. There was linearity of response to varying concentrations for all compounds, as well as good reproducibility from repeat injections. Other compounds could also be measured using this particular HPLC system (e.g. tryptophan, tyrosine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, DOPAC, xanthurenic acid, 5-hydroxytryptophan, DOPA, adrenaline etc.). These could be optimally separated by manipulation of the mobile phase characteristics (e.g. % methanol or pH change) or by altering the potential of the detector or by omitting the octylsulphonate (which acts as an

ion-pairing agent to increase the retention of some compounds, in particular, fast-eluting species).

2.3.4 Calculation of concentrations (ng/g tissue):

'Direct' method

Ratios of sample peak height to standard peak height were adjusted to allow for the concentration of standard used, dilution factor and weight of tissue.

Example calculation:

$$\frac{\text{HVA in sample}}{\text{HVA in standard}} \times 50 \text{ ng/ml} \times 4 \times \frac{0.5 \text{ (+ weight) ml}}{\text{weight (g)}}$$

= ng/g tissue

Extraction method

Extracted ratios of compound in sample to internal standard in sample were compared to similar ratios in standards, and corrected to allow for the weight of tissue used.

Example calculation:

$$\frac{\text{DA in sample/DHBA in sample}}{\text{DA in standard/DHBA in standard}} \times 10 \times \frac{1}{\text{weight}}$$

= ng/g tissue

2.4 Determination of choline acetyltransferase (CAT) activity

CAT activity in post mortem brain in HD and controls (frontal and temporal cortex, hippocampus and caudate) was determined using our modification of

the radioenzymatic method of Fonnum (1975). This involved the measurement of tritium-labelled acetylcholine (ACH) production from the enzymatic (CAT) conversion of tritiated acetyl CoA and choline.

$$\text{Acetyl CoA}^{*} + \text{choline} \xrightarrow{\text{CAT}} \text{acetylcholine}^{*} + \text{CoA}$$

(* = radiolabel)

Eserine was present during the incubation to inhibit the cholinesterase activity. Tetraphenylboron (TPB) was used for the extraction of acetylcholine* into a toluene-based scintillant, resulting in a two phase solution which could have the radioactivity measured using a liquid scintillation counter.

The method was as follows:

Tissue (approx. 50 mg) was homogenised on ice using a glass/glass homogeniser in 0.25 ml 0.1 M phosphate-buffered saline (PBS, pH 7.5) containing 0.4% vol/vol Triton X-100, 1 mM EDTA and 428 mM sodium chloride. The homogenate was transferred and a further 250 μl PBS used to wash the tube. The pooled PBS homogenate was centrifuged for 10 minutes at 50000 g at 4°C. At regular timed intervals, 50 μl supernatant was mixed and incubated at 37°C with 50 μl prewarmed "hot" working buffer containing 8 mM choline, 0.1 mM eserine 0.2 mM labelled acetyl CoA (hot/cold) in PBS. This was done in triplicate for each sample in a scintillation vial insert. After a timed period (5 or 15 minutes) the reaction was stopped by adding and mixing ice-cold phosphate buffer (PB) (0.01 M pH 7.4) and removal from the water bath, onto ice. Freshly made (1 ml) TPB (2 g/l in acetonitrile) was added with 3 ml toluene

based scintillation fluid and mixed. The radioactivity/tube was measured using liquid scintillation counting. Blanks were made using PBS in the place of the tissue supernatant. Activities were compared with specific activity standards prepared by adding 50 μl "hot" working buffer to 3 ml aqueous based scintillation fluid, in the presence of 300 μl water to aid mixing. Two time points (5 and 15 minutes) were measured for each sample, because investigation of the time course of the reaction showed a greatly increased surge during the first two minutes, resulting in a lack of linearity. To avoid this factor, 10 minute activity was determined by subtracting the 5 minute value from the 15 minute value, and corrected to produce a value/hour.

2.4.1 Example calculation:

$$\frac{(\text{sample} - \text{blank})15 - (\text{sample}-\text{blank})5}{\text{specific activity standard}} \times \text{ACH} \times \frac{\text{time}}{\text{weight}}$$

= μmole ACH produced/hour/g tissue

Good linearity was demonstrated from a curve produced from diluting homogenate samples. There was good stability of samples except for caudate, which needed further dilution (1+9), which made it more susceptible to degradation during assays. Thus assays of shorter duration were used for caudate.

The small volume in a large scintillation vial insert was thought to be introducing variability, as the liquid drop was difficult to mix, therefore a microassay was tried resulting in better reproducibility. Plastic microtubes (1.2 ml) were used to incubate the assay. After stopping the reaction, the contents were poured into a

scintillation vial insert and the TPB was used to wash out the microtube into the scintillation vial insert, which was then counted as usual.

2.5 Determination of concentrations of amino acids

Recent HPLC methods for the measurement of amino acids involve pre-column derivatisation followed by isocratic separation and ED. Two modified methods were used here to determine concentrations of GABA, glutamate and aspartate in human post mortem brain tissue (Pearson et al., 1991).

2.5.1 Method A)

The first method (Allison et al., 1984) used tert-butylthiol and o-phthalaldehyde (OPA) as a reagent to produce relatively stable consistent derivatives. The method (modified by Czudek, personal communication) was used to measure concentrations of GABA and glutamate.

Brain tissue (50-100 mg) was homogenised in 0.5 ml 0.1 M PCA containing 100 μM ascorbate. The homogenate was centrifuged at 12000 g for 3 minutes. An aliquot of supernatant was derivatised and a further aliquot was used for the concurrent measurement of monoamines and their metabolites in a separate HPLC system. Reagent was prepared by dissolving 54 mg OPA in 1 ml ethanol and adding 80 μl t-butylthiol and 8.9 ml PB (0.1 M pH 9.5). Refrigerated stock solutions of amino acids (2 mg/ml) were diluted daily to 100 μg/ml using methanol/water (50/50 vol/vol). 20 μl standard solution or sample supernatant was reacted with 200 μl reagent for 4 minutes at room temperature. This was shown to produce a maximal reaction producing

derivatives which were stable for at least 30 minutes. A 40 µl aliquot of derivative was diluted with a 25-fold excess of mobile phase and then injected into the HPLC system. This involved the isocratic separation of amino acid derivatives at 40°C using a reverse phase HPLC-ED. The system comprised of a 25 cm X 4.6 mm Spherisorb ODS 5 µm column with a BAS detector containing a glassy carbon electrode set at +0.7 V (vs. Ag/AgCl) for quantification. The mobile phase consisted of PB (0.1 M pH 5.8) containing 0.05 mM EDTA and 52% methanol. Concentrations were determined using sample peak height compared with standard peak height, and correcting for dilution and weight.

2.5.2 Example calculation:

$$\frac{\text{glutamate (sample)}}{\text{glutamate (standard)}} \times 100\ \mu g/ml \times \frac{(0.5 + \text{weight})}{\text{weight}}$$

= µg/g tissue

This method was used successfully for about 1 year (providing much of the data in this thesis), but proved to have some limitations. Careful timing of derivatisation was necessary and derivatives decomposed noticeably after approximately 30 minutes. Moreover the use of t-butylthiol was banned in the institution, due to a noxious smell, together with a high volatility. Therefore an alternative approach had to be found.

2.5.3 Method B)

The second method used was modified by us from Jacobs (1987) which used inorganic sulphite instead of thiol, to produce stable OPA derivatives of primary amines suitable for ED. Our new method replaced the 80 µl t-butylthiol in the previously

described reagent with 1 ml 1 M sodium sulphite. This has now been routinely used for several years in this laboratory, simply producing clean, stable, odourless and reproducible derivatives (Pearson et al., 1991).

Tissue was prepared as described for the previous method. If required, dilution with 4 volumes 0.1 M disodiumtetraborate buffer (pH 9.5) permitted storage frozen at -20°C, for later analysis. ß-aminobutyric acid (BABA, 2 mg/ml) was used as an internal standard as this was similar in structure to GABA, although it was shown not to co-elute with any sample peaks. Refrigerated stock solutions (2 mg/ml) were dissolved using water (with a few drops of sodium hydroxide where necessary), and diluted weekly to 100 µg/ml with 0.1 M PCA. BABA (20 µl) was added to 800 µl standard or sample supernatant and 100 µl of this mixture was reacted with 160 µl reagent for 20 minutes. The reagent was made by dissolving 54 mg OPA in 1 ml ethanol and adding 1 ml 1 M sodium sulphite and 18 ml 0.1 M borate buffer (pH 9.5). This reagent was stable at room temperature for several weeks. The derivative (40 µl) was diluted 20-fold with water and 50 µl was injected into the HPLC system. This was a similar system to that described earlier, except an ODS-2 5 µm column was used with mobile phase at pH 5.6 and containing 5% methanol. Here the detector was set at +0.85 V for optimal response.

Amino acid concentrations were determined by the calculation of ratio of peak height to internal standard in samples, compared to the equivalent ratio in standards. This was corrected for sample dilution and weight to give a concentration (µg/g tissue).

2.5.4 Example calculation:

$$\frac{\text{glutamate (sample)}}{\text{BABA}} \times 100 \times \frac{(0.5 + \text{weight})}{\text{weight}}$$

$$\frac{\text{glutamate (standard)}}{\text{BABA}}$$

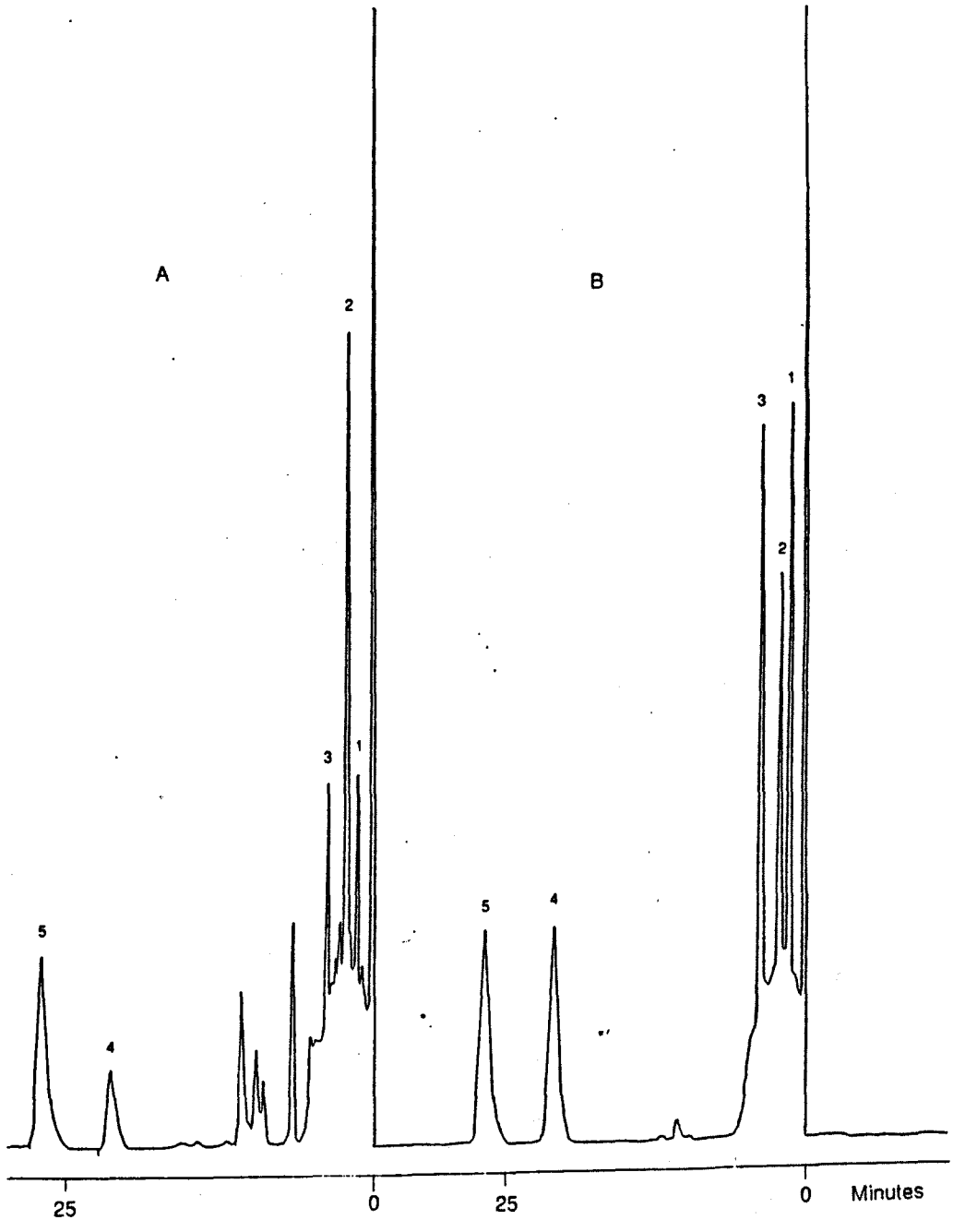
= µg/g tissue

The chromatogram (Fig. 2.1) shows a typical standard and a typical sample. Single peaks (retention times in minutes) were demonstrated for aspartate (1.4), glutamate (2.2), glycine (3.8), GABA (21.0) and BABA (26.7). The peaks in the samples were assigned after comparison of retention times with those from standard solutions, and by confirmation of their identical co-elution by observing the expected summation of peak heights of standards added to samples. Percentage recoveries from added standards were aspartate: 99 ± 6, glutamate: 98 ± 4, GABA: 100 ± 5 (mean ± SD n = 5). Reproducibility was tested using eight samples from two brain tissue homogenates which yielded coefficients of variation of 3% for aspartate, 6% for glutamate and 7% for GABA. Linear standard curves for concentration (0-150 µg/ml) versus response were demonstrated for all the amino acid derivatives.

Figure 2.1

HPLC chromatogram of amino acid derivatives

A shows a typical brain sample; B shows a typical standard (100 μg/ml). Peaks are in order: aspartate, glutamate, glycine, GABA and BABA (full scale deflection = 500nA).



The derivatives were shown to be stable for more than five hours when kept on ice. Borate-diluted samples were found to be stable on storage at -20°C; this was demonstrated in samples frozen for several weeks that yielded for glutamate: 98.8 ± 8%; aspartate: 100 ± 11%; GABA: 97 ± 10% (mean ± SD n = 6) of the values obtained from freshly prepared homogenates of the same tissue. These results also demonstrate the low variability between different preparations from the same sample of dissected tissue. Values of frozen samples kept for several months were shown to be within their expected range. The optimal time of derivatisation was tested using 4 minute increments; 20 minutes derivatisation was shown to produce a maximal response. Brain amino acids were typically in the range of 100-1000 μg/g tissue, although much greater sensitivity could be achieved by eliminating the dilution stages of the method. Here the limit of detection for GABA was 0.5 pmol on the column, although this sensitivity was not possible for aspartate and glutamate due to their proximity to the solvent front. If GABA determination was not required, the resolution of glutamate and aspartate could be improved by increasing the retention time by reducing the methanol content/adjusting the pH of the mobile phase; this would decrease sample throughput. Similarly if GABA alone were required, its retention time could be diminished. This would make the method more applicable to the determination of GABA in dialysates from the recently-developed in vivo brain dialysis techniques (Ungerstedt, 1984).

Other amino acids also giving single peaks (not shown) were asparagine, threonine, glutamine and arginine. Three amino acids produced a co-eluting peak: histidine, taurine and alanine. Using these conditions no peaks were identified for tyrosine,

lysine, tryptophan, methionine, phenylalanine, cystine, valine, isoleucine, cysteine, leucine or homocysteine within a 30 minute run time (Mercer, personal communication 1988). However the three aromatic acids of neurochemical importance as neurotransmitter precursors could be measured using a modified buffer to increase the elution rate. Thus for optimal separation of tryptophan, phenylalanine and tyrosine, a mobile phase of pH 5.7 and containing 22% methanol was used yielding retention times (relative to BABA:100) of 664, 555 and 127 respectively.

The sulphite method described is the final protocol used, where many factors have been optimised. Most problems were solved although some (e.g. the "lump" in the solvent front) could not be eliminated from method. A time course for derivatisation, showed a maximal response at 20 minutes (at room temperature), producing derivatives that were stable for up to 4 hours if kept at 4°C. If not kept cold, the stability was reduced to 1 hour, particularly for derivatives of GABA. Originally mobile phase was used for the final dilution of the derivatives, however, water was demonstrated to increase both the response and the stability. Alkaline conditions for the reaction were described by Jacobs (1987) as optimal so pH 9.5 borate buffer was used, whereas Jacobs suggested that the mobile phase needed a pH greater than 5-6. We used a mobile phase of pH 5.6 which was a compromise between maximal response and separation, particularly of the fast-eluting aspartate and glutamate which Jacobs predicted would be difficult to determine without an ion-pairing agent. We tried using dodecyltetramethylammonium bromide, but this resulted in a high interfering solvent front.

Many parameters were changed in an effort to reduce or remove the "lump" in the solvent front, that was even present in blanks and standards and thus was inherent in the derivatisation reaction. We tried varying reagent:sample ratio, OPA concentration and sulphite concentration; using fresh reagents, different column, HPLC grade water for all solutions, different temperature for the HPLC system, testing for the presence of ammonia, altered pump speed of the HPLC system, different containers for the reaction and diluting the derivative more after the reaction. Reducing the OPA concentration, diluting the reagent more and increased dilution post-derivatisation were the only parameters which had a decreasing effect and these were incorporated into the method. However further reducing the OPA concentration compromised the linearity of response and thus could not be continued.

2.6 Statistics and validation

Statistical comparisons were generally made by using the Student's t-test of data, logarithmically transformed to achieve a normal distribution. Where there were only very small groups, the non-parametric Mann Whitney U-test was employed. Pearson correlation coefficents were used.

All the methods used were validated by comparison with authentic standard solutions, and in some cases an internal standard. Also, standards added to samples were determined to assess recovery and losses attributable to the method. The precision and linearity of response to varying concentrations were verified, by the determination of replicate samples and standards.

Chapter 3

Neurotransmitter results and discussion

3.1 Results

Our results from the major study of neurotransmitters and their metabolites in Huntington's disease (HD) and controls can be seen in Tables 3.1 - 3.8 (Reynolds and Pearson, 1987a).

3.1.1 γ-aminobutyric acid

From Table 3.1 it can be seen that there is a significant decrease in γ-aminobutyric acid (GABA) concentrations throughout the whole brain in HD, including areas of the basal ganglia, s.nigra, limbic system and cortex. The caudate, putamen, and lateral pallidum show the greatest deficits (about 65% loss), whereas the medial pallidum is slightly less affected (59% loss). Even in the cortex there are GABA losses ranging from 29-37%. There was no overlap, at all, of HD and control ranges in the basal ganglia. Interestingly, there was no significant correlation between striatal GABA concentrations and duration of disease (results not shown).

3.1.2 Glutamate

Table 3.2 shows significant deficits of glutamate in the HD brain in the caudate, putamen, hippocampus, frontal and temporal cortex; however, in the pallidum and s.nigra concentrations are significantly increased above control values. The greatest losses of glutamate can be seen to occur in the caudate and putamen (losses of 32% and 24% respectively).

Table 3.1

GABA concentrations in the brain in Huntington's disease

Values are means ± s.d. in μg/g tissue. Ranges in parentheses. *p < 0.05, **p < 0.01 ***,p < 0.001

Region	Controls	Huntington's disease
Caudate	314 ± 89.8 (232 - 563)	100 ± 40.3*** (40 - 195)
Putamen	461 ± 61.7 (366 - 571)	165 ± 52.1*** (70 - 352)
Medial pallidum	783 ± 66.7 (638 - 924)	318 ± 105*** (127 - 545)
Lateral pallidum	744 ± 112 (522 - 994)	241 ± 92.7*** (116 - 452)
Substantia nigra (compacta)	405 ± 177 (207 - 797)	209 ± 138*** (70 - 736)
Hippocampus	196 ± 55.0 (124 - 334)	139 ± 43.0*** (68 - 247)
Amygdala	224 ± 37.8 (156 - 288)	193 ± 57.6** (68 - 381)
Frontal cortex (BA 10)	196 ± 37.1 (119 - 267)	140 ± 36.8*** (65 - 223)
Temporal cortex (BA 21)	203 ± 27.9 (147 - 250)	128 ± 32.4*** (36 - 194)

Table 3.2

Glutamate concentrations in the brain in Huntington's disease

Values are means ± s.d. in μg/g tissue. Ranges in parentheses. *p < 0.05, **p < 0.01, ***p < 0.001

Region	Controls	Huntington's disease
Caudate	1694 ± 219 (1327 - 2344)	1137 ± 384*** (444 - 2058)
Putamen	2160 ± 272 (1631 - 2848)	1638 ± 397*** (702 - 2570)
Medial pallidum	749 ± 139 (383 - 1104)	1114 ± 541** (468 - 3442)
Lateral pallidum	885 ± 179 (554 - 1191)	1009 ± 218* (581 - 1473)
Substantia nigra (compacta)	1001 ± 146 (788 - 1294)	1275 ± 319*** (682 - 1884)
Hippocampus	1232 ± 166 (692 - 1556)	1082 ± 298** (406 - 1698)
Amygdala	1616 ± 172 (1314 - 2089)	1586 ± 402 (707 - 2426)
Frontal cortex (BA 10)	1507 ± 272 (1041 - 2128)	1366 ± 407* (628 - 2281)
Temporal cortex (BA 21)	1252 ± 139 (993 - 1522)	1048 ± 286*** (475 - 1645)

3.1.3 Dopamine

Dopamine (DA) concentrations in the HD brain (Table 3.3) are seen to be significantly increased in the putamen (48% increase), lateral pallidum (198% increase) and medial pallidum (471% increase), compared with control values. Significant losses were identified in the s.nigra and hippocampus and values in the amygdala and cortex were not significantly changed from normal.

3.1.4 Homovanillic acid

Significant losses of homovanillic acid (HVA) were evident (Table 3.4) in the HD brain throughout the basal ganglia, with the caudate showing the greatest deficit of 48% and the medial pallidum being relatively less affected (loss of 24%). However, in both regions of the cortex, HVA concentrations were significantly increased (by 72-151% compared with control values).

3.1.5 5-Hydroxytryptamine

Table 3.5 demonstrates the increased concentrations of 5-hydroxytryptamine (5HT) observed throughout the HD brain (except for the hippocampus, which was not significantly changed). Significance is achieved in the basal ganglia, amygdala and temporal cortex, with the lateral pallidum showing the greatest effect (increase of 129%) and the amygdala the least effect (increase of 24%).

Table 3.3

Dopamine concentrations in the brain in Huntington's disease

Values are means ± s.d.in ng/g tissue. Ranges are in parentheses. *p < 0.05, **p < 0.01, ***p < 0.001

Region	Controls	Huntington's disease
Caudate	2667 ± 694 (1410 - 4571)	2645 ± 2300* (108 - 12133)
Putamen	4125 ± 1176 (466 - 6899)	6093 ± 2844* (1488 - 13048)
Medial pallidum	96.6 ± 76.1 (21 - 299)	554 ± 531*** (22 - 2143)
Lateral pallidum	976 ± 662 (55 - 2908)	2918 ± 2171*** (137 - 6166)
Substantia nigra (compacta)	649 ± 267 (369 - 1617)	574 ± 357* (18 - 1517)
Hippocampus	21.6 ± 18.6 (7 - 80)	14.9 ± 13.0* (2 - 67)
Amygdala	48.5 ± 35.8 (6 - 133)	75.0 ± 77.2 (3 - 297)
Frontal cortex (BA 10)	2.0 ± 1.4 (0.4 - 5)	2.0 ± 1.4 (1 - 7)
Temporal cortex (BA 21)	5.9 ± 4.7 (1.2 - 22)	10 ± 9.5 (1 - 41.9)

Table 3.4

Homovanillic acid concentrations in the brain in Huntington's disease

Values are means ± s.d. in ng/g tissue. Ranges in parentheses. *p < 0.05, **p < 0.01, ***p < 0.001

Region	Controls	Huntington's disease
Caudate	4401 ± 1194 (2332 - 7130)	2306 ± 1101*** (810 - 5262)
Putamen	9538 ± 2600 (4658 - 17638)	6176 ± 2158*** (2855 - 11895)
Medial pallidum	3855 ± 1343 (2103 - 7864)	2937 ± 896** (1513 - 5941)
Lateral pallidum	6110 ± 1674 (3580 - 10928)	4440 ± 1450*** (1583 - 8063)
Substantia nigra (compacta)	5217 ± 1092 (3313 - 7409)	3920 ± 1080*** (1641 - 5424)
Hippocampus	333 ± 111 (128 - 607)	310 ± 160 (122 - 925)
Amygdala	865 ± 200 (569 - 1354)	818 ± 352 (308 - 1853)
Frontal cortex (BA 10)	61.4 ± 19.9 (34 - 118)	154 ± 78.8*** (41 - 289)
Temporal cortex (BA 21)	124 ± 33.6 (64 - 202)	213 ± 102*** (72 - 465)

Table 3.5

5-Hydroxytryptamine concentrations in the brain in Huntington's disease

Values are means ± s.d in ng/g tissue. Ranges in parentheses.
*p < 0.05, **p < 0.01, ***p < 0.001

Region	Controls	Huntington's disease
Caudate	219 ± 60.7 (97 - 339)	328 ± 221* (81 - 1017)
Putamen	320 ± 75.7 (147 - 435)	632 ± 216*** (151 - 1094)
Medial pallidum	245 ± 80.8 (117 - 492)	458 ± 179*** (145 - 928)
Lateral pallidum	224 ± 53.9 (147 - 350)	514 ± 219*** (141 - 1141)
Substantia nigra (compacta)	671 ± 170 (399 - 1210)	813 ± 322 (288 - 1566)
Hippocampus	117 ± 82.8 (36 - 471)	95.1 ± 48.7 (14 - 225)
Amygdala	233 ± 57.5 (138 - 366)	290 ± 112* (46 - 569)
Frontal cortex (BA 10)	11.8 ± 5.2 (6 - 28)	20.3 ± 18.1** (5 - 98)
Temporal cortex (BA 21)	13.8 ± 6.9 (8 - 35)	18.1 ± 8.6 (4 - 36)

3.1.6 5-Hydroxyindoleacetic acid

Changes in concentrations of 5-hydroxyindoleacetic acid (5HIAA) in the HD brain can be seen in Table 3.6. A significant increase is obvious throughout the brain (except again the hippocampus, which was not significantly changed). Here, the putamen shows the greatest increase (by 135%) and the s.nigra and amygdala the smallest increase (by 30%).

3.1.7 Noradrenaline

Noradrenaline (NA) concentrations (Table 3.7) in the HD brain were significantly increased in pallidal regions (by 115 and 257%), whereas in limbic regions and the temporal cortex there were significant decreases (20% losses) compared with control values.

3.1.8 Choline acetyltransferase

Table 3.8 demonstrates the significant deficits observed in choline acetyltransferase (CAT) activity in the HD brain. The caudate showed a decrease of 60% and the hippocampus and frontal cortex showed losses of 31% and 12% respectively.

Table 3.6

5-Hydroxyindoleacetic acid concentrations in the brain in Huntington's disease

Values are means ± s.d in ng/g tissue. Ranges in parentheses.
*p < 0.05, **p < 0.01, ***p < 0.001

Region	Controls	Huntington's disease
Caudate	441 ± 139 (189 - 813)	762 ± 367*** (217 - 2182)
Putamen	794 ± 227 (352 - 1151)	1864 ± 724*** (803 - 3596)
Medial pallidum	1195 ± 261 (905 - 1767)	2021 ± 789*** (870 - 4608)
Lateral pallidum	995 ± 132 (692 - 1214)	2015 ± 748*** (937 - 3848)
Substantia nigra (compacta)	2241 ± 672 (1348 - 3876)	2857 ± 1080** (1332 - 5839)
Hippocampus	331 ± 99.7 (98 - 569)	362 ± 176 (132 - 1027)
Amygdala	566 ± 102 (385 - 750)	742 ± 295** (346 - 1756)
Frontal cortex (BA 10)	64.7 ± 17.8 (42 - 102)	117 ± 45.8*** (45 - 219)
Temporal cortex (BA 21)	89.9 ± 21.3 (58 - 142)	153 ± 59.6*** (42 - 291)

Table 3.7

Noradrenaline concentrations in the brain in Huntington's disease

Values are means ± s.d. in ng/g tissue. Ranges in parentheses. *p < 0.05, **p < 0.01, ***p < 0.001

Region	Controls	Huntington's disease
Caudate	-	-
Putamen	-	-
Medial pallidum	36.0 ± 20.9 (14 - 102)	77.4 ± 52.1*** (14 - 223)
Lateral pallidum	31.9 ± 36.1 (2 - 134)	114 ± 96.8*** (4 - 446)
Substantia nigra (compacta)	-	-
Hippocampus	33.4 ± 16.6 (13 - 87)	25.6 ± 27.8*** (4 - 94)
Amygdala	95.5 ± 51.3 (31 - 141)	71.3 ± 52.0** (7 - 205)
Frontal cortex (BA 10)	6.3 ± 3.1 (1 - 13)	5.5 ± 2.6 (2 - 13)
Temporal cortex (BA 21)	7.0 ± 3.2 (2.2 - 13)	5.8 ± 3.3* (0.1 - 15.5)

Table 3.8

Choline acetyltransferase activity in the brain in Huntington's disease

Values are means ± s.d. in μmol/h/g tissue. Ranges in parentheses. *p < 0.05, **p < 0.01, ***p < 0.001

Region	Controls	Huntington's disease
Caudate	5.5 ± 2.0 (0.5 - 8.4)	2.2 ± 1.8*** (0.01 - 6.2)
Hippocampus	1.04 ± 0.42 (0.59 - 2.42)	0.72 ± 0.42*** (0.14 - 1.65)
Frontal cortex (BA 10)	0.34 ± 0.06 (0.22 - 0.60)	0.30 ± 0.10* (0.14 - 0.47)
Temporal cortex (BA 38)	0.38 ± 0.06 (0.22 - 0.46)	0.34 ± 0.11 (0.14 - 0.57)

3.2 Discussion

3.2.1 GABA

Our results are consistent with and confirm the well-established (Perry et al., 1973; Spokes et al., 1980; Urquhart et al., 1975) losses of GABA in the basal ganglia in HD, and indicate that these deficits extend to all nigral, limbic and cortical areas measured. While pallidal regions both show a loss, there is a differential effect with the medial part exhibiting a smaller loss than the lateral. This is consistent with an observation by Spokes (1980) that there is a loss of of glutamic acid decarboxylase activity in the lateral, but no significant change in the medial part of the HD pallidum. Further evidence comes from work by Reiner et al. (1988) suggesting that in HD the inhibitory GABAergic striatal-pallidal pathways are lost, but that relatively more degeneration occurs to the lateral than to the medial. As the disease advances, further losses of the medial pathway occur altering the medial/lateral balance, thought to result in the initial choreiform movements being replaced by more rigid symptoms.

The identified losses of GABA can be further understood in association with previously reported losses of neuropeptides in HD (Emson, 1986; Beal et al., 1988b), including substance P, met-enkephalin and dynorphin. Albin et al. (1990a,b) has suggested that in HD, the striatal (enkephalin-containing) projections to the lateral pallidum and those projecting to the s.nigra (containing substance P) are relatively lost compared to the striatal-medial pallidum pathway (containing substance P). This is again consistent with our observation of differential pallidal losses. Furthermore, concentrations of met-enkephalin are reduced in the

whole pallidum and s.nigra and there are losses of substance P, again in the whole pallidum and s.nigra (Emson, 1986).

Another report (Ellison et al., 1987) suggested that there are gradients of GABA deficits (caudate > putamen) and (external > internal pallidum), with which our findings are consistent, although in their study the hippocampus and frontal cortex (BA 10) did not exhibit significant losses. Pathological changes, with gradients of losses, have been reported to parallel these neurochemical changes (Vonsattel et al., 1985), in particular the losses of striatal spiny neurones.

Spokes et al. (1980) and Ellison et al. (1987) did not find the significant cortical or hippocampal losses that we identified, however Urquhart et al. (1975) reported general cortical losses which reached significance in the occipital cortex. Similarly, Perry et al. (1973) reported losses in occipital and temporal cortex, as did Gramsbergen et al. (1986), who also reported losses evident in the s.nigra. These cortical losses in HD are very similar to those identified in another neurodegenerative disorder, Alzheimer's disease, and there has been speculation as to whether these cortical GABA deficits are related to cognitive changes seen in HD patients (see section 5.3).

Deficits in GABA concentrations maybe a reflection degenerating GABAergic neurones, or perhaps a change in GABA regulation and turnover. However, indications from the measurement of GABA uptake sites in HD (defined by radio-labelled nipecotic acid binding) may provide relevant evidence, as GABA terminals can be identified which are unlikely to be altered by changes in metabolism. The determination

of GABA uptake sites in the putamen in a small subgroup of the HD series (Czudek and Reynolds, 1990) revealed a deficit which was parallelled by the losses of GABA concentrations. This was also reported by another study (Simpson et al., 1988). These findings suggest actual GABAergic neurodegeneration is occurring in HD. This view is also consistent with other measures of GABAergic terminals, including reduced GAD activity and immunohistochemical neuropathological markers of GABAergic neuronal losses (Spokes et al., 1979, 1980; Reiner et al., 1988). Further evidence supporting a GABAergic deficit are reports of reduced GABA binding in HD (Lloyd et al., 1980).

It is unlikely that external factors can explain the deficits of GABA concentrations identified in HD. Differences between the groups were minimised and GABA is reportedly unaffected by postmortem delay, apart from an initial increase (Spokes et al., 1979). Also GABA concentrations are unaffected by agonal state, unlike previous measures of GABAergic activity such as GAD activity (Spokes, 1979), so the unavoidable difference in cause of death between the groups (HD patients generally die of bronchopneumonia, whereas most of the controls died suddenly) is not a causative factor here. Another difference is the male/female ratio, as the control group is predominantly male (reflecting the low incidence of sudden death in females of this age range). There was no effect of sex difference on GABA concentrations in the HD group. It is always important to consider drug effects, especially as almost all of the HD patients were taking neuroleptics or tetrabenazine. However, we have found that such treatment has no effect on GABA concentrations in HD brain (see section 3.4); certainly concentrations of GABA in striatal and

pallidal regions are reportedly not significantly changed from control values in neuroleptic-treated schizophrenic patients (Toru et al., 1988).

3.2.2 Glutamate

The significant losses of glutamate in the HD striatum, hippocampus and cortex (frontal and temporal) have largely been unreported previously, except for in the striatum (Perry et al., 1982; Gramsbergen et al., 1986), but neither group found cortical losses. However, the recent results of Ellison et al. (1987) are consistent with our findings. They found reduced striatal glutamate concentrations and cortical losses, significant in four areas: frontal (BA 9), premotor (BA 6), postcentral (BA 1,2,3) and occipital (BA 17). The losses in BA 10 and BA 21 appeared to be not significant and the hippocampus was not significantly changed, in contrast to our findings. Also, Kremzner et al. (1979) found a glutamate deficit in the frontal cortex. A notable effect was obvious in the pallidum and s.nigra, where we found a significant increase in concentrations of glutamate, the effect being the strongest in the medial pallidum. Here too, the results of Ellison et al. (1987) match ours: they also demonstrated increased concentrations in the pallidum (medial > lateral) and reaching significance in the s.nigra (pars compacta).

In this case too, the interpretation of any changes in glutamate concentrations is complex, as glutamate is not solely a neurotransmitter and plays a vital role in metabolism in the brain. Thus any changes may be due to metabolic differences, or could be reflecting glutamatergic neuronal losses. Alternatively, losses of GABAergic neurones may

consequently produce changes in turnover of glutamate. Evidence regarding the nature of the losses comes from work by Fonnum (1984). He suggested that lesioning glutamate pathways produces losses of glutamate concentrations (20-45%), whereas lesioning GABA pathways, such as the striatonigral, has no effect on glutamate concentrations in the s.nigra. Thus our results show regions with increases or decreases of glutamate concentrations, together with deficits in GABA, suggesting that they are not directly related. Other evidence regarding the nature of the changes in glutamate concentrations comes from studies of glutamate uptake sites (defined by D-aspartate binding) in the HD brain (Cross et al., 1986c). They demonstrated losses of glutamate uptake sites in the caudate (72% loss), putamen (60% loss) and hippocampus (32% loss), but no significant change in the one area of cortex examined (temporal). This suggests an actual loss of glutamatergic neurones in these areas. Another putative (but unproven) marker for glutamate terminals is the presence of ornithine decarboxylase, thought to be found predominantly in glutamate terminals (Wong et al., 1982). Their study showed significant losses of this enzyme in the HD striatum and losses in the cortex (significant only in frontal and parietal). Further evidence for a reduced glutamatergic system comes from receptor studies showing a deficit in the binding to NMDA, kainate and quisqualate receptors in HD (Greenamyre et al., 1985; Young et al., 1988; Beaumont et al., 1979).

There is evidence that glutamate is a neurotransmitter in the corticostriatal tract (Fonnum et al., 1981), and also that glutamate uptake predominantly occurs in the glutamatergic corticostriatal tract (Henn and Hamberger, 1971).

Thus, the deficits of striatal glutamate that we have identified are consistent with degeneration of the corticostriatal tract. Furthermore, there is evidence suggesting that layer V of the cortex is atrophied, and this is the origin of the corticostriatal fibres (Bruyn et al., 1979). Ellison et al. (1987) showed that striatal lesioning causes secondary degeneration of the glutamatergic corticostriatal pathway, however, there was no deficit of glutamate concentrations. Therefore, they suggested that in HD there must be a primary degeneration of glutamatergic neurones, reflected by the reduced glutamate concentrations in the cortex.

The increases found in the pallidum and s.nigra are less expected. There is evidence to suggest a glutamatergic pathway from the subthalamic nucleus to the pallidum (Robertson et al., 1989) which is important in the balance between the medial and lateral pallidum. Thus, any differential increase may result in the medial part becoming relatively less active producing choreiform movements (Crossman et al., 1989). Increases in concentrations of glutamate may just be reflecting general tissue atrophy, producing a relative increase, as the pallidum shrinks by as much as the striatum (Lange et al., 1976).

Cortical losses of amino acids have been described in other dementing disorders (Rossor et al., 1984; Sasaki et al., 1986; Reynolds and Warner, 1988) suggesting a possible common feature, however, we have shown no association between these cortical deficits of amino acids and the dementia of HD (Reynolds et al., 1990a) (see section 5.3).

Here too, as with the GABA data, it is important to eliminate any potential factors or group difference,

which may have contributed to the changes observed. There is no effect of agonal state (Perry et al., 1982), post mortem delay (Perry et al., 1981) or prior drug treatment (see section 3.4). There was a small sex effect (males glutamate concentration = 1750 μg/g / females = 1522 μg/g), which may be related to the longer average duration of disease (males = 11.0 years/ females = 13.7 years) producing greater deficits. However, these slight differences are unlikely to be responsible for the substantial deficits identified.

Glutamate has also been suggested to be a neurotoxin and was of potential interest in the aetiology of HD (McGeer and McGeer, 1976a) (see section 6.2). However, our findings in HD do not support the hypothesis suggesting an increased glutamatergic system.

3.2.3 5HT and 5HIAA

In the HD brain, our results demonstrate a substantial increase of 5HT and the metabolite 5HIAA, in all examined regions other than the hippocampus, in which there was no significant change. One previous report (Bernheimer and Hornykiewicz, 1973) showed striatal 5HT not to be significantly changed, but this study only used 3 or 4 cases. Otherwise this system was little studied in HD until a recent report by Kish et al. (1987). Their group found generally elevated concentrations of 5HT throughout the putamen (81% increase) and caudate (30-40% increase) which is consistent with our results (increases of 98% and 50% respectively). Furthermore, these changes were parallelled by increased 5HIAA concentrations in both studies. Kish also found no significant effect in the nucleus accumbens, no significant effect in the s.nigra and

did not examine the cortex.

5HT uptake sites (defined by radio-labelled paroxetine binding) were shown by Cross et al. (1986c) to be increased in the striatum (significantly in the putamen). Thus, the increases we have shown are consistent with an increase in striatal serotonergic terminals. Whether this increase is absolute or relative remains unclear, as it may solely reflect tissue atrophy. Kish reported that the lack of significant effect in the nucleus accumbens and s.nigra was evidence for the latter, as these regions undergo little atrophy in HD, however, in our hands the s.nigra shows a 20% increase in concentrations of both 5HT and 5HIAA. In the temporal cortex, at least there appears to be a greater increase of 5HIAA compared with 5HT, suggesting that here there is an increase in turnover. One hypothesis is that this may be reflecting a difference in tryptophan metabolism, as tryptophan concentrations are increased in the HD cortex (see kynurenine chapter). Other recent evidence (Kalen et al., 1989) suggests that GABA may have a tonic inhibitory effect on serotonergic raphé neurones via its influence on the lateral habenula-dorsal raphe pathway. Thus, GABA losses such as those we have identified in HD, may increase 5HT turnover and release. It has been suggested that most serotonergic cortical axons innervate GABAergic neurones (Tork et al., 1988) and thus our data are consistent with possible altered regulation of these systems in HD. Furthermore, subcortical receptor losses (5HT 1) have been reported in HD (Cross et al., 1986b; Waeber et al., 1989), but they found no significant cortical losses.

The consequences of such serotonergic increases are not clear. 5HT has been reported to have a possible

role in the production of myoclonus and hyperactivity (Gerson and Baldesserini, 1980). Furthermore, 5HT or 5HIAA have been implicated in depression, aggression and dementia, but usually associated with decreased serotonergic activity (Palmer et al., 1988; Kurlan et al., 1988; Peyser and Folstein, 1990).

As with the other measures, any group differences must be considered. There was no effect of sex difference or post mortem delay. However, agonal state has been shown to affect 5HT and 5HIAA concentrations (Gottfries et al., 1974). As terminal hypoxia correlates with reduced concentrations, this is unlikely to be a causative factor because the HD group (with more hypoxic death) has the higher 5HT and 5HIAA values. There was no effect of neuroleptics, but prior treatment with tetrabenazine is known to deplete monoamines (Pearson and Reynolds, 1988) (see section 3.4), including 5HT which argues against it causing the increases found in HD.

3.2.4 DA and HVA

In the past the nigrostriatal pathway was thought to be relatively spared in HD, and responsible for the hyperkinetic movement disorder, but previous reports of DA concentrations in the HD brain have been inconsistent, thus providing no real evidence. Suggestions have included an increase in the putamen (Melamed et al., 1982), caudate (Bernheimer and Hornykiewicz, 1973), both (Spokes, 1980) or neither (Reynolds and Garrett, 1986; Bird and Iversen, 1974). Also, increased concentrations have been described in the s.nigra and the nucleus accumbens (Spokes, 1980). Thus our results from this study are predictably consistent with one of the reports.

In our hands, DA was increased (by 48%) in the putamen but with no significant increase in the caudate or the s.nigra. Kish et al. (1987) recently reported reduced concentrations of DA in both the putamen and the caudate, and postulated that the discrepencies between the various studies were due to differences in post mortem delay. In particular they suggested that the study by Spokes (1980) had a control group which was artefactually low, because they had a mean post mortem delay 10 hours longer than their HD group. The converse would be predicted for our series, as the controls were 12 hours shorter than the HD group. The Kish groups were closer matched. However, Reynolds and Garrett (1986) demonstrated that there was much greater variability of DA concentrations in the HD group, which they suggested had contributed to the inconsistencies reported; individual values were described as falling well below and well above the control range. Our own results concurred with this, thus, in the caudate although the means were identical, the HD range (108-12133 ng/g) was obviously not similar to the controls (1410-4571 ng/g). The identified increase of DA concentrations in both parts of the pallidum is consistent with the results of Spokes (1980). Areas, other than the basal ganglia (such as the cortex) have not been determined in these studies. One factor that may explain some of the variability, is prior drug treatment. Neuroleptics have been reported not to affect DA concentrations in the striatum (Shannak and Hornykiewicz, 1980; Crow et al., 1979), whereas, tetrabenazine has been shown to deplete monoamines (Pearson and Reynolds, 1988) (see section 3.4), especially striatal DA (loss of 2/3). Thus the HD group appears to predictibly have a sub-group with low striatal DA concentrations.

The functional activity of the DA system must be considered to understand the changes observed, therefore HVA concentrations were determined. We found significant losses throughout, except for the hippocampus and amygdala and in fact in the cortex there were significant increases. These findings are consistent with results from Reynolds and Garrett (1986) and Kish et al. (1987), although Melamed et al. (1982) found no change in their study. Tyrosine hydroxylase (the rate-limiting enzyme in the formation of DA) activity is reportedly unchanged in the HD striatum (McGeer and McGeer, 1976b) and D1 and D2 receptors are decreased in the striatum (Seeman et al., 1989; Joyce et al., 1988; Cross et al., 1983), athough the reported deficit in the pallidum (De Keyser et al. 1989a,b) has been challenged and in our hands there was no significant change (Reynolds et al., 1990b). Therefore, evidence would appear to support the suggestion that there is a down-regulation of the nigrostriatal dopaminergic system in HD, which still remains intact.

HVA concentrations are unaffected by post mortem delay and neuroleptics (Crow et al., 1979) and only slightly reduced by tetrabenazine (see section 3.4), indicating that these factors are not responsible for the substantial deficits seen in the HD brain. There was no effect of age, sex and agonal state on the measurements of either DA or HVA concentrations.

What these changes mean in the disease profile is unclear. Within the basal ganglia, dyskinesia may be predicted from such changes, although there is evidence implicating GABAergic losses in the pallidum in the production of choreiform movements (Pearson et al., 1990). An altered DA system in areas of the brain other than the basal ganglia, may

be producing psychiatric symptoms. It is reported that there are limbic dopaminergic and GABAergic changes occurring in schizophrenia (Reynolds, 1989).

3.2.5 Acetylcholine

We observed the well established loss of CAT activity in the striatum in HD (Bird and Iversen, 1974; Aquilonius et al., 1975; McGeer and McGeer, 1976b; Spokes, 1980). Our results showed a significant 60% decrease in caudate and 30% loss in the hippocampus, which is consistent with Spokes (1980) who demonstrated significant losses in the caudate, putamen, hippocampus and to a lesser extent in the nucleus accumbens. However, he did not find any significant pallidal or cortical changes, whereas we found a significant decrease in the frontal cortex only. McGeer and McGeer (1976b) reported "low but variable CAT activity in some areas of the cortex and patchy losses in the basal ganglia". Bird and Iversen (1974) described "a subgroup of normal CAT activity range" in the striatum within the HD group, as well as a group severely affected with significant CAT activity deficits, the loss being unrelated to clinical symptoms. Here too they supported the "patchy loss" description, but as the caudate appeared to correlate with the putamen a "low activity tissue patch" was thought to be unlikely. These CAT activity deficits are consistent with a significant loss of cholinergic receptors in the putamen in HD, although there was no significant change in the cortex. These changes of neurochemical cholinergic markers may reflect a down regulation of the cholinergic system resulting in reduced antagonism of the nigrostriatal dopaminergic pathway. The cortical deficit appears to be be unassociated with the severity of dementia, as was the striatal

deficit (see section 5.3). These findings are contrary to those seen in Alzheimer's disease and Parkinson's disease (Rossor et al., 1984; Perry et al., 1983). It would seem that in HD cortical cholinergic projections are not lost to the same extent as in these other disorders.

3.2.6 NA

The increased pallidal concentrations of NA concentrations in HD that we observed are consistent with the study of Spokes (1980), whereas the deficits in limbic and temporal cortex are novel findings. Pallidal noradrenergic receptors were also decreased in HD (Enna et al., 1976a,b) which is also consistent. However, the effects of these pallidal changes are difficult to predict, although NA and DA may have synergistic influences. The limbic and cortical deficit in HD would be expected to be associated with psychiatric symptoms (e.g. dementia or affective disorder). However, in HD there was no such relationship between these clinical symptoms and NA concentrations (see section 5.3).

3.3 Cortical changes in HD

There has been a long-running debate regarding the involvement of the cortex in HD (Reynolds and Pearson, 1987b). The question remains as to whether there are cortical neuronal losses and if so, which specific neurone populations are affected. There is a view that cortical changes are not particularly obvious when compared at a gross neuropathological level (Richardson, 1990; Vonsattel et al., 1985; Hallervorden, 1957). However, there is now strong evidence supporting the concept of global cortical losses (of 20%) independent of striatal changes or

severity of grade of HD (Bruyn et al., 1979; Cudkowicz and Kowall, 1990; de la Monte et al., 1988; Lange, 1981). Particular layers (3,5 and 6) apppear to be affected and frontal (and prefrontal) pyramidal projection neurone populations have been reported to show degeneration which may, or may not be associated with severity of the disease (Bird and Coyle, 1986; Cudkowicz and Kowall, 1990; Sotrel and Myers, 1990). Evidence of cortical atrophy has also been described using imaging techniques in HD. The study of Starkstein et al. (1988, 1989) used computed tomography (CT) in HD and reported cortical atrophy that correlated with age, but not with measures of subcortical atrophy. Positron emission tomography (PET) studies have also revealed cortical hypometabolism in HD (Kuwert et al., 1990; Weinberger et al., 1989). At the moment, single photon emission computed tomography and magnetic resonance imaging investigations have not reported measures of the cortex (Reid et al., 1988; Simmons et al., 1986).

The aim of our study was to determine any neurochemical cortical changes, in order to identify and understand the specific profile of neuronal losses. We examined samples from frontal (BA 10) and temporal (BA 21) cortex, as part of our comprehensive regional distribution investigation in the brain in HD.

We found (see section 3.2) significant losses of GABA and glutamate concentrations, together with significantly increased concentrations of HVA, 5HT, 5HIAA and 3-hydroxykynurenine in the cortex in HD. Tryptophan concentrations were significantly increased in the temporal cortex and CAT activity was decreased in the frontal cortex, whereas, concentrations of quinolinic acid (QA) (see section

6.7), DA and NA were not significantly changed. In a blind test of the data it was easily possible to differentiate the HD cases from the controls, using decreased cortical GABA and glutamate concentrations as the criteria. Our finding of decreased levels of cortical GABA was consistent with the studies of Perry et al. (1973), Gramsbergen et al. (1986) and Urquhart et al. (1975), but contrary to Spokes et al. (1980) and Ellison et al. (1987) that reported no significant cortical changes. Substance P has been shown to be increased in cortical regions, as have neuropeptide Y (NPY), somatostatin (SS) and NADPH-diaphorase in some areas. As substance P co-localises with NPY or SS in some cortical GABAergic neuronal populations, this provides evidence for selective sparing. However, cholecystokinin has been shown to be decreased in the cortex, defining an alternative subpopulation of GABAergic neurones; those which are lost in HD (Hays et al., 1981). This would be consistent with the cortical GABA deficit that we have identified. As described earlier, a further subpopulation of GABAergic neurones are innervated by serotonergic axons (Tork et al., 1988), and the absence of cortical 5HT receptor losses suggests that these may also be spared in HD.

The cortical glutamate deficit that we identified in HD was also reported by Ellison et al. (1987) and Kremzner et al. (1979), but not found in the studies of Perry et al. (1973) or Gramsbergen et al. (1986) and glutamate uptake sites were found to be not significantly changed (Cross et al., 1986c). The deficit is consistent with the described losses of frontal cortex pyramidal projection neurones, which are likely to be glutamatergic. However, in the temporal cortex the glutamate deficit may be reflecting intrinsic glutamate losses, not solely

projection neurones showing retrograde losses, as postulated by Sotrel and Myers (1990). Further evidence in support of glutamatergic losses is the atrophy of cortical layer 5 in HD (thought to be the origin of the corticostriatal glutamatergic pathway).

Our results show, for the first time, increased concentrations of 5HT and 5HIAA in the cortex in HD. This may merely be a reflection of cortical atrophy resulting in a relative increase, but in the temporal cortex at least there appears to be increased turnover of 5HT. As discussed earlier (see section 3.2), most cortical serotonergic axons innervate GABAergic neurones, therefore it is possible that there is altered regulation of these transmitters. The increased tryptophan and 3HK concentrations (see section 7.8) are consistent with dysfunction of tryptophan metabolism generally, even in the cortex, although QA concentrations were not significantly altered (see section 6.7).

The CAT activity deficit in one cortical region was examined to identify any association with the dementia occurring in HD, similar to the changes seen in dementia of Alzheimer's or Parkinson's diseases. However, we showed cortical neurochemical parameters (including monoamines and amino acids) to be unassociated with dementia in HD (see section 5.3). Moreover, the only correlates with the dementia were caudate glutamate and GABA concentrations. These findings are consistent with PET and CT studies showing caudate (and not cortical) measures to be related to the dementia in HD (see section 5.3). Further studies of psychiatric symptoms have correlated depression in HD with greater cortical atrophy and hypometabolism in the prefrontal cortex, whereas apathy in HD was

linked with hypometabolism of the cingulate gyrus (Peyser and Folstein, 1990). In Alzheimer's disease, aggression has been reported to correlate with reduced 5HT concentrations in the orbital gyrus (Palmer et al., 1988) and in schizophrenics, there are reports of increased cortical glutamatergic markers and turnover of dopamine (Reynolds, 1989; Deakin et al., 1989).

The increased HVA concentrations in the cortex in HD are a novel finding. Further examination of the data suggests a bimodal distribution in HD, with a 'normal HVA' group within control range, and a 'high HVA' group. Furthermore, high HVA is found to be associated with increased glutamate concentrations, and normal HVA with decreased concentrations of glutamate ($p < 0.001$ in BA 21). There was no correlation in the control group, and no association with GABA concentrations, neuroleptics or tetrabenazine treatment. One possible explanation for this abnormal distribution may be that in HD the striatal degeneration results in increased cortical HVA. However, with glutamatergic losses, this HVA increase may not occur. One consequence of treatment with long-term neuroleptics is increased HVA concentrations in the frontal cortex (Bowers and Hoffman, 1986) (due to a mesocortical feedback mechanism, that could be GABAergic or glutamatergic). In HD, we found no association of cortical HVA and prior neuroleptic treatment, thus it would seem that the controlling influence is lost in HD. Furthermore, another result of long-term neuroleptics is an initial increase followed by a chronic decrease of striatal HVA concentrations (perhaps mediated by long-loop GABA feedback). As there is also no relationship between striatal HVA and neuroleptics in HD, this feedback loop also seems to be lost in HD. Thus, the usual peripheral

effects (increased HVA in the CSF and plasma) with neuroleptic treatment, would be predicted not to occur in HD. Indeed, studies by Caraceni et al. (1977) or Mattson and Persson (1973) indicated no changes of CSF HVA concentrations in HD, after neuroleptic treatment.

3.4 The effects of prior treatment with tetrabenazine

At present there is no drug treatment that can prevent the overall progressive decline of HD, however, individual symptoms can be ameliorated to some extent. The hyperkinetic movements can be reduced by the use of drugs that block DA receptors (e.g. phenothiazines, butyrophenones) or drugs that deplete the storage of DA (tetrabenazine, reserpine). Tetrabenazine and reserpine have a similar action as monoamine-depleting agents. Tetrabenazine causes reversible depletion of storage granules, is relatively fast-acting and of short duration, compared with reserpine which causes longer-term depletion of storage granules (Jankovic, 1982). Both drugs can produce side effects such as drowsiness, anxiety and depression, which are more severe with reserpine. This would suggest that it is not solely the nigrostriatal DA system which is affected. Thus, tetrabenazine is the drug of choice for the suppression of chorea (Jankovic, 1982). Previous animal studies have shown that tetrabenazine depletes the three monoamine neurotransmitters DA, NA and 5HT (Lane et al., 1976). Post-mortem studies provide a unique opportunity to assess the neurochemical effects of tetrabenazine in man, as its actions have so far only been studied clinically or in the experimental animal.

Here we discuss such an investigation using results from sub-groups of the large series of patients with HD and from control subjects (described in section 2.1). Two groups were obtained after assessment of drug histories: those known to have been taking tetrabenazine at death and those known never to have taken tetrabenazine. Several different regions were studied (caudate, putamen, medial and lateral pallidum, amygdala, hippocampus, s.nigra pars compacta, temporal and frontal cortex). Concentrations of DA, NA, 5HT, 5HIAA, HVA, glutamate and GABA and activities of CAT were analysed statistically using the Student's t-test of logarithmically transformed data. Details of the two groups can be seen in Table 3.9.

The results (Pearson and Reynolds, 1988) in Table 3.10 show that the mean concentrations of monoamine neurotransmitters were less in almost all regions of the brain studied in the tetrabenazine-treated group, except for the medial pallidum and DA in the frontal cortex. This depletion reached significance for DA (in the striatum, hippocampus and s.nigra), NA (in the limbic regions) and 5HT (in the s.nigra and frontal cortex). HVA concentrations were also reduced in all areas, but not significantly (results not shown). 5HIAA, glutamate and GABA concentrations were unchanged, with no trend to lower levels apparent (Table 3.11). The activity of CAT was also similar in both groups (results not shown). There were no significant group differences in age, sex, post-mortem delay, neuroleptic treatment or duration of disease.

Table 3.9

Details of subjects providing brain tissue post mortem : tetrabenazine

Values are means ± s.d.

	Age (Years)	Sex	Post mortem (Hours)	Duration (Years)
Tetrabenazine-treated	59 ± 9	5M 6F	40 ± 26	12 ± 4
Tetrabenazine-free	51 ± 15	3M 4F	41 ± 23	8 ± 4

Table 3.10

Monoamine neurotransmitters in the brain in Huntington's disease following tetrabenazine (TBZ) treatment

Values are means ± s.d. in ng/g tissue. *p < 0.05, **p < 0.01

	Noradrenaline	Dopamine	Serotonin
Caudate			
TBZ-treated	-	1113 ± 993**	213 ± 128
TBZ-free	-	3281 ± 2089	340 ± 261
Putamen			
TBZ-treated	-	4019 ± 3013*	550 ± 208
TBZ-free	-	7358 ± 2101	644 ± 278
Lateral pallidum			
TBZ-treated	88.5 ± 105	1797 ± 1697	426 ± 182
TBZ-free	101 ± 81.8	3456 ± 1867	604 ± 323
Medial pallidum			
TBZ-treated	55.5 ± 41.2	457 ± 536	425 ± 133
TBZ-free	55.7 ± 45.0	379 ± 408	401 ± 198
Amygdala			
TBZ-treated	47.1 ± 40.2*	59.4 ± 95.7	222 ± 100
TBZ-free	105 ± 77.3	73.0 ± 62.3	306 ± 162
Hippocampus			
TBZ-treated	14.1 ± 10.2*	9.6 ± 7.2*	78.9 ± 48.7
TBZ-free	47.4 ± 54.5	22.2 ± 14.4	93.8 ± 43.6
Nigra (compacta)			
TBZ-treated	-	324 ± 220*	631 ± 300*
TBZ-free	-	610 ± 186	954 ± 225
Frontal cortex			
TBZ-treated	5.2 ± 2.4	2.4 ± 1.8	13.0 ± 6.6*
TBZ-free	6.6 ± 2.2	2.2 ± 1.4	39.7 ± 37.6
Temporal cortex			
TBZ-treated	5.5 ± 4.8	6.4 ± 4.2	15.9 ± 10.7
TBZ-free	6.2 ± 1.8	8.1 ± 7.0	20.4 ± 8.5

Table 3.11

Amino acid neurotransmitters in the brain in Huntington's disease following tetrabenazine (TBZ) treatment

Values are means ± s.d. in ug/g tissue.

	GABA	Glutamate
Caudate		
TBZ-treated	90 ± 33	994 ± 333
TBZ-free	111 ± 46	1168 ± 580
Putamen		
TBZ-treated	166 ± 38	1613 ± 246
TBZ-free	167 ± 75	1683 ± 748
Lateral pallidum		
TBZ-treated	221 ± 58	1000 ± 191
TBZ-free	282 ± 135	983 ± 330
Medial pallidum		
TBZ-treated	318 ± 81	1067 ± 333
TBZ-free	297 ± 104	867 ± 371
Amygdala		
TBZ-treated	182 ± 56	1518 ± 346
TBZ-free	178 ± 61	1552 ± 554
Hippocampus		
TBZ-treated	138 ± 39	1087 ± 279
TBZ-free	144 ± 34	995 ± 303
Nigra (compacta)		
TBZ-treated	185 ± 105	1236 ± 329
TBZ-free	184 ± 55	1148 ± 333
Frontal cortex		
TBZ-treated	141 ± 37	1370 ± 595
TBZ-free	137 ± 16	1275 ± 251
Temporal cortex		
TBZ-treated	136 ± 27	1038 ± 219
TBZ-free	126 ± 53	965 ± 376

The present findings provide direct evidence for the effects of tetrabenazine on neurotransmitters in the human brain. The results confirm previous studies in animals (Lane et al., 1976), demonstrating the depletion of monoamines in brain tissue, caused by treatment with tetrabenazine. A decrease of approximately 40% of DA, NA and 5HT has been reported in whole brain of rats, with glutamate, aspartate and acetylcholine also decreased, but to a lesser extent. The present results show a general depletion of monoamines which was more prominent in specific areas for particular neurotransmitters. The greatest reduction was that of DA in the caudate (34% of control value), which may have a role in the ameliorative effect of tetrabenazine on chorea. There appeared to be no compensatory increase in the turnover of DA, as concentrations of HVA were also reduced, although not significantly. However, a far greater decrease in HVA below control levels has been shown in most regions of the brain in HD (see section 3.2), indicating a deficit of DA associated with the disease process.

The significant depletion of monoamines in the limbic system shows that tetrabenazine was not solely affecting areas of the brain associated with movement. It is possible that the depletions caused by tetrabenazine in the limbic system result in side effects, such as depression. Certainly deficits of monoamines have been implicated in the aetiology of affective disorders (van Praag, 1977). Also, it has been found that the effects of tetrabenazine are antagonised by tricyclic antidepressants and monoamine oxidase inhibitors (Jankovic, 1982), which increase synaptic concentrations of monoamine neurotransmitters, again providing clinical evidence for depletion of monoamines caused by tetrabenazine.

There were regional differences in the depletion of monoamine caused by tetrabenazine. Regional variations were also observed in animal studies, which may reflect either differences in turnover, or alternatively differing sensitivity of vesicular storage to tetrabenazine (Pettibone et al., 1984).

Along with its effect on the storage of monoamines, tetrabenazine has also been shown to have a blocking effect on DA receptors (Reches et al., 1983). The ability of tetrabenazine to induce acute dystonia (Burke et al., 1985) has been described as clinical evidence of its blocking properties. Thus, the dual action of tetrabenazine on DA systems has been proposed to be the basis of its efficacy in the treatment of hyperkinetic movement disorders. Whether this antagonist action is important clinically is unclear, since the ameliorative effects of and perhaps some of the side effects can be understood in terms of the depletions of monoamine transmitters that have been identified here.

Chapter 4

Neurochemical correlates of chorea

4.1 Introduction

The aim of this part of the study was to attempt to define any neurochemical correlates of the chorea in HD.

Chorea is a hyperkinesia characterised by random, involuntary jerky fragments of movements and it has been postulated that ballism is an extreme form of chorea, with a common mechanism (Crossman et al., 1984; Dewey and Jankovic, 1989). An amelioration of choreiform movements in HD can be achieved using dopamine blocking/depleting drugs, possibly as a result of reduced inhibition of the striatal GABAergic efferents. The nigrostriatal dopaminergic pathway was initially thought to be relatively spared, however our own results (see section 3.2) and previous evidence (Reynolds and Garrett, 1986) suggest that a reduction in dopamine turnover occurs in HD. How these changes relate to the symptom of chorea in HD is unclear since, despite the massive neuronal losses in the striatum, experimental models in the primate have been unsuccessful in producing chorea by striatal lesions (Crossman et al., 1988).

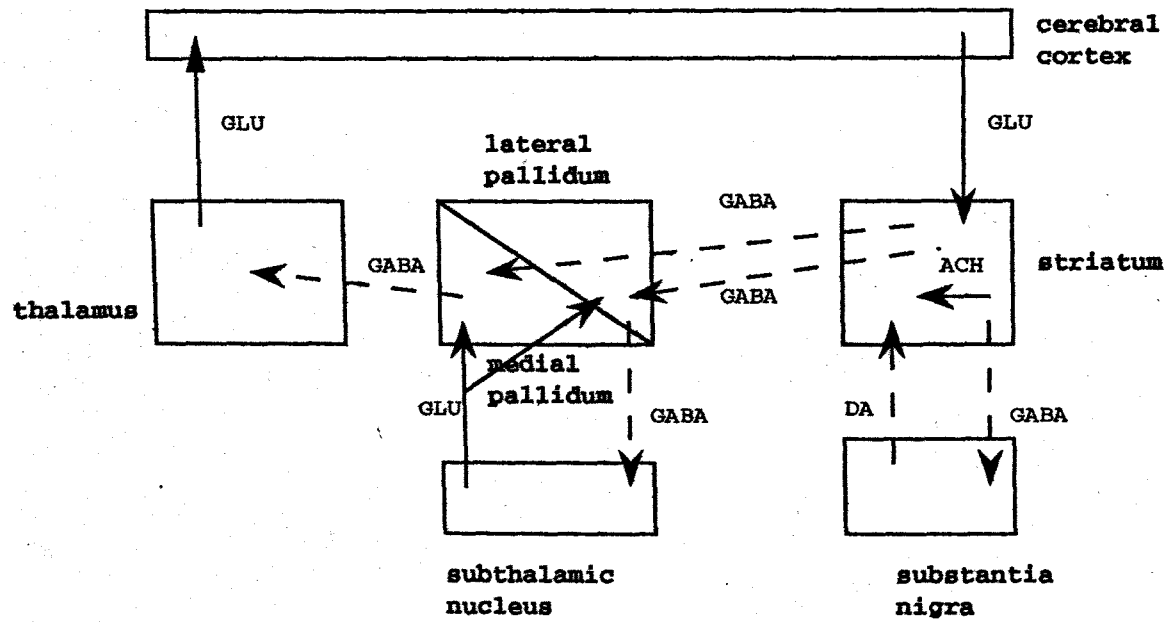


Figure 4.1 Simplified scheme for neuronal connections of the striatum

Excitatory

Inhibitory

Current ideas implicate GABAergic losses in the choreiform movements in HD, especially in pathways of the pallidum, subthalamic nucleus (STN) and thalamus (see Fig. 4.1). Lesioning the STN in primates is known to produce ballism consistently, as does injection of a GABA antagonist into the STN. The result of both situations is a loss of STN activity (as demonstrated by reduced uptake of 2-deoxyglucose). Injection of the GABA antagonist, bicuculline, into the lateral pallidum produced chorea, but similar injections into the striatum were not found to result in chorea, except when diffusion into the lateral pallidum occurred (Crossman et al., 1988). Injection of kynurenic acid (a glutamate receptor antagonist) into the medial pallidum produced chorea presumably due to the blockade of the excitatory glutamatergic pathway from the STN to the medial pallidum (Robertson et al., 1989). Thus the relative balance of the different parts of the basal ganglia would appear to be crucial to the understanding of chorea.

4.2 Methods

Case histories from the HD series (n = 46) were assessed by an experienced neurologist (Dr.Kenneth Heathfield) to determine the degree of chorea. A global impression from the medical and nursing case notes, in the 42 cases where there was sufficient information, provided approximate gradings: mild, moderate and severe. Thus 'mild' described cases in which the chorea was limited in site and extent, whereas 'severe' were the most greatly affected and were profoundly restricted in their mobility. Recognising that some lack of precision is inevitable in such a retrospective assessment, only the two extreme groups were used for comparison although data from the intermediate moderate group

are also shown. Age, sex, post mortem delay and duration of disease are shown in Table 4.1. Since in almost all of the small groups the distribution of the parameters under investigation did not approximate to normality, non-parametric Mann Whitney U-tests were employed for statistical comparison. In each sub-group, concentrations of γ-aminobutyric acid (GABA), glutamate, dopamine (DA), homovanillic acid (HVA) and 5-hydroxytryptamine (5HT) were determined from the putamen, caudate and medial and lateral pallidum.

4.3 Results

The results (Pearson et al., 1990) in Table 4.2 illustrate the significant losses of GABA in all parts of the basal ganglia (see section 3.2). Both pallidal regions show significant deficits in GABA concentrations, although the medial shows a smaller loss than the lateral. Furthermore GABA is found to be less decreased in severely choreic as opposed to mildly choreic patients. Although also evident in the lateral pallidum and striatal regions, the effect is only significant in the medial pallidum.

We found increased concentrations of DA (see section 3.2) in the putamen in HD , which was found to a greater extent in both lateral and medial parts of the pallidum, whereas a significant decrease in the concentration of HVA was found in all areas of the basal ganglia. There was no difference between DA (Table 4.3) or HVA (Table 4.4) concentrations in mildly and severely choreic in any of the areas studied. However, DA in the moderate group was shown to be significantly increased in both the lateral pallidum and caudate; only when compared to severe group, but not with the mild group. A further observation is that concentrations of

glutamate (Table 4.5) in the caudate were shown to be significantly increased in the severe, as compared with the mild group. There were no significant changes relating the degree of chorea with 5HT concentrations (Table 4.6).

Table 4.1

Details of subjects providing brain tissue post mortem: chorea

Values are means. Ranges in parentheses.

	Age (Years)	Sex	Post mortem (Hours)	Duration (Years)
Controls	57 (21-82)	24M 3F	31 (3-79)	-
Huntington's disease	56 (30-83)	23M 23F	43 (3-153)	12 (3-24)
Mild	44 (30-69)	5M 4F	57 (46-72)	12 (3-20)
Moderate	58 (32-72)	7M 9F	35 (3-153)	12 (5-23)
Severe	58 (43-75)	6M 10F	42 (8-120)	13 (4-24)

Table 4.2

Concentrations of GABA in the basal ganglia: chorea

Values are means ± s.d. in μg/g tissue.
*p < 0.05 versus severe group, ***p < 0.001 versus controls

	Medial pallidum	Lateral pallidum	Putamen	Caudate
Controls	783 ± 67	744 ± 112	461 ± 62	314 ± 90
Huntington's disease	318 ± 105***	241 ± 93***	165 ± 52***	100 ± 40***
Mild	247 ± 75*	203 ± 81	148 ± 66	83 ± 49
Moderate	312 ± 108	250 ± 169	169 ± 40	116 ± 45
Severe	339 ± 90	227 ± 78	169 ± 59	94 ± 24

Table 4.3

Concentrations of dopamine in the basal ganglia: chorea

Values are means ± s.d. in ng/g tissue.
*p < 0.05 moderate versus severe group
***p < 0.001 HD versus controls

	Medial pallidum	Lateral pallidum	Putamen	Caudate
Controls	97 ± 76	976 ± 662	4125 ± 1176	2667 ± 694
Huntington's disease	554 ± 531***	2918 ± 2171***	6093 ± 2844***	2645 ± 2300*
Mild	334 ± 257	2588 ± 1720	6079 ± 3247	2438 ± 2205
Moderate	713 ± 552	3886 ± 2603*	6828 ± 3052	3562 ± 2924*
Severe	514 ± 617	2154 ± 1643	5300 ± 2729	2032 ± 1801

Table 4.4

Concentrations of homovanillic acid in the basal ganglia: chorea

Values are means ± s.d. in ng/g tissue.
**p < 0.01, ***p < 0.001 HD versus controls

	Medial pallidum	Lateral pallidum	Putamen	Caudate
Controls	3855 ± 1343	6110 ± 1674	9538 ± 2600	4401 ± 1194
Huntington's disease	2937 ± 896**	4440 ± 1450***	6176 ± 2158***	2306 ± 1101***
Mild	2968 ± 931	4158 ± 1261	5760 ± 2787	1963 ± 1419
Moderate	3150 ± 1046	5022 ± 1720	6405 ± 1891	2757 ± 1281
Severe	2616 ± 660	3940 ± 1199	5818 ± 1878	2049 ± 767

Table 4.5

Concentrations of glutamate in the basal ganglia: chorea

Values are means ± s.d. in μg/g tissue.
*p < 0.05 mild versus severe group
**p < 0.05, ***p < 0.001 HD versus controls

	Medial pallidum	Lateral pallidum	Putamen	Caudate
Controls	749 ± 139	885 ± 179	2160 ± 272	1694 ± 219
Huntington's disease	1114 ± 541**	1009 ± 218**	1638 ± 397***	1137 ± 384***
Mild	1001 ± 281	1017 ± 248	1542 ± 489	970 ± 460*
Moderate	1042 ± 374	962 ± 206	1503 ± 351	1130 ± 446
Severe	1260 ± 798	1021 ± 205	1706 ± 314	1108 ± 240

Table 4.6

Concentrations of 5-Hydroxytryptamine in the basal ganglia: chorea

Values are means ± s.d. in ng/g tissue.
*p < 0.05, ***p < 0.001 HD versus controls

	Medial pallidum	Lateral pallidum	Putamen	Caudate
Controls	245 ± 80.8	224 ± 53.9	320 ± 75.7	219 ± 60.7
Huntington's disease	458 ± 179***	514 ± 219***	632 ± 216***	328 ± 221*
Mild	501 ± 276	573 ± 231	690 ± 297	290 ± 259
Moderate	490 ± 168	563 ± 191	678 ± 200	420 ± 285
Severe	410 ± 111	458 ± 241	593 ± 186	281 ± 131

4.4 Discussion

4.4.1 Moderate group

Data from the moderate group shows a significant change in DA levels in two areas compared with the severe group. However, although the results have been shown, it was felt that valid conclusions could not be made due to the lack of precision from retrospective assessment of the degree of chorea. The two extreme groups could be presumed to be strongly defined, whereas there was potentially much overlap with the intermediate group. Thus, further conclusions will only be drawn from the mild and severe results.

4.4.2 Monoamines in chorea

The present results (see section 3.2) provide no evidence for an increased activity of the dopamine system in HD, in fact the substantial losses of HVA indicate reduced turnover of this system. The lack of any consistent association between the degree of chorea and striatal or pallidal DA or HVA suggests that this system is unrelated to the production of choreiform movements. Similarly there appears to be a lack of connection between 5HT and choreiform movements.

4.4.3 Amino acids in chorea

The well-established loss of GABA in HD (see section 3.2) is obvious here. While pallidal regions show significant losses, the medial part shows a smaller loss than the lateral, which may result in the medial/lateral GABAergic balance being disturbed. This is consistent with results from Spokes (1980) that demonstrate a loss of glutamate decarboxylase,

the synthetic enzyme for GABA, in the lateral but not the medial pallidum in HD. Furthermore, our study shows GABA to be less decreased in severely choreic patients than in those with only mild chorea, this being significant only in the medial pallidum. Although perhaps counter-intuitive, such findings are wholly consistent with the primate models of chorea that implicate a relative underactivity of the medial pallidum, demonstrated by decreased uptake of 2-deoxyglucose (Crossman et al., 1988). In HD the inhibitory GABAergic striatal-pallidal pathways are lost, but relatively more degeneration occurs to the lateral than to the medial pathway, which are functionally separate having different peptidergic cotransmitters (Albin et al., 1989). Thus the medial pallidum is underactive, relative to the lateral part, which results in decreased inhibition of the venteroanterior (VA) and venterolateral (VL) thalamus via its GABAergic projection. The lateral pallidum is relatively overactive which results in greater inhibition of the STN via its GABAergic projection. As a result the excitatory glutamatergic pathway from the STN to the medial pallidum is also underactive, compounding the effect in the medial pallidum. This combination of effects indicates that there is an overactivity of the VA/VL thalamus, which mediates the striatal outputs to the motor cortex. Presumably, further degeneration of the GABAergic innervation of the medial pallidum will result in a disinhibition of this structure, counteracting, to some extent, the effect described above. Thus, as the disease progresses, the initial choreiform movements become increasingly more akinetic and rigid, as the eventual degeneration of this pathway follows the initial loss of lateral pallidal GABAergic innervation (Reiner et al., 1988).

4.5 Factors influencing GABA changes

It is important to consider the many factors which could be artefactually affecting the GABA changes, although a regionally specific effect argues against external influence. The control group contains fewer females than the HD group (as does the mild compared with severe group), but this cannot account for the large changes observed (Reynolds and Pearson, 1987a). The minor differences in postmortem delay in the HD subgroups would not be expected to affect the results, since GABA concentrations are reportedly not significantly affected by this factor (Perry et al., 1981). GABA is similarly unaffected by the cause of death (Spokes et al., 1979); moreover bronchopneumonia was the major cause of death in both the HD groups. Several of the patients studied had received neuroleptic drugs, however we have found that such treatment has no significant effect on GABA concentrations in the HD brain (see section 3.2); certainly concentrations of GABA in striatal and pallidal regions are reported to be not significantly changed from control values in neuroleptic-treated schizophrenic patients (Toru et al., 1988). Further clinical factors may be of relevance; one such factor is rigidity. Within the mild chorea subgroup, five of the cases were described as displaying rigidity, whereas none of the severe group had this symptom. Nevertheless, the remainder of the mild subgroup also showed the same trend in GABA losses. Another factor is age, however, any effect of age would predict a decrease of GABA in the older subgroup (the severe subgroup) which is contrary to our findings (Spokes et al., 1979). An associated factor here is age at onset of the disease. In particular, two patients in the

mild subgroup were younger onset cases (displaying symptoms before age 20). However, removal of these two cases from the subgroup had no effect on the overall results. Nor can this difference be ascribed to duration of disease, which exhibited no correlation with pallidal GABA concentrations, nor any significant difference between the two groups.

The only other significant finding was the relative increase in caudate glutamate concentrations in the severe subgroup, compared with the mild. This is consistent with a relative loss of corticostriatal neurones, perhaps reflecting a temporal effect between mild and severe chorea. Again, all the above factors discussed in relation to GABA must be considered (see section 3.2). A specific glutamate change would not be predicted by differences in agonal state (Perry et al., 1982), duration of disease, sex, age, postmortem delay (Reynolds and Pearson, 1987a), drugs, rigidity or age at onset. One factor which may be relevant is the degree of dementia (see section 5.3), as in HD, dementia is associated with deficits of GABA and glutamate in the caudate. Thus, if the mild chorea subgroup were relatively more demented this would predict the observed change in concentrations of glutamate in the caudate. Also evident is a corresponding increase in caudate GABA concentrations, although this is not significant. However, there is insufficient clinical information regarding the degree of dementia of the cases in these subgroups.

Chapter 5

Neurochemical correlates of dementia

5.1 Introduction

Huntington's disease (HD) is not just a hyperkinetic movement disorder. Clinically, there is a wide spectrum of symptoms including psychiatric changes and a decline in cognitive function, which may precede the first signs of chorea.

There has been considerable speculation regarding the dementia of HD, specifically, comparing it with Alzheimer's disease (AD) and Parkinson's disease (PD). It has been suggested that HD and PD dementias are subcortical and that Alzheimer's disease (AD) is cortical in origin (Foster, 1986; Cummings and Benson, 1984). "Subcortical dementia" was first described in 1974 by Albert et al. using progressive supranuclear palsy as an example. Clinical symptoms included forgetfulness, slowness of thought processes, the inability to manipulate acquired knowledge, apathy, depression and altered personality. The characteristics of HD dementia, reported by McHugh and Folstein (1975) parallelled this subcortical dementia model, and were inconsistent with the dementia of AD (where aphasia, agnosia, amnesia and intellectual impairment are prevalent). However, an alternative view is that "such classification is an unsubstantiated oversimplification and the integration of individual subcortical projection systems, with the cortex must be understood" (Whitehouse et al., 1986; Chui, 1989).

In HD there is considerable atrophy of the basal

ganglia (50% loss) in all areas, including the pallidum, reflecting the neurodegeneration occurring. Limbic and cortical areas are also atrophied (Lange et al., 1976; de la Monte et al., 1988), although the degree to which the deficits in different regions are involved in the production of psychiatric symptoms and dementia is unclear. Studies have been made to determine neurochemical markers for dementia. One consistent finding is an associated loss of cholinergic neurones (demonstrated by measuring choline acetyltransferase (CAT) activity). Using post mortem cortical tissue from patients with AD, there was an inverse correlation between cognitive impairments and other measures of dementia with CAT activity (Mountjoy et al., 1984). A similar equivalent reduction of cortical CAT activity was also reported in demented PD patients, even without neuropathological signs of D (Perry et al., 1983). There was no evidence of similar investigations of CAT activity in dementia in HD, so our study aimed to determine whether any such association with a cholinergic deficit was indicated.

5.2 Methods

We investigated brain tissue taken post mortem from the large series of HD patients and matched controls. Dementia was rated on a four point scale (absent, mild, moderate and severe) by an experienced neurologist (Dr. Kenneth Heathfield) using a global assessment of clinical medical and nursing records.

Table 5.1

Details of subjects providing brain tissue post mortem: dementia

Values are means ± s.d. *p < 0.05 versus severely demented cases.

	Age (years)	Sex	Duration (years)
Controls	57 ± 14	24M 3F	-
Huntington's disease	56 ± 12	23M 24F	12 ± 6
Not demented	67 ± 10*	3M 2F	14 ± 4
Severely demented	54 ± 12	5M 7F	14 ± 4

Only the two extreme groups, absent (n=5) or severe (n=12) were investigated due to the approximation of such a retrospective study (Table 5.1). These were almost equivalent to points 1 and 6/7 on the Global Deterioration Scale of Reisberg et al. (1982). CAT activity was determined in three cortical areas (temporal cortex, frontal cortex and hippocampus) and in the caudate. Concentrations of γ-aminobutyric acid (GABA), glutamate, 5-hydroxytryptamine (5HT) and noradrenaline (NA) were also measured in striatal and cortical regions of the brain tissue. Statistical comparisons were made using t-test of logarithmically-transformed data.

5.3 Results and Discussion

Reduced striatal CAT activity has been well established in HD (Spokes, 1980; McGeer and McGeer, 1976b) and indeed our own results (Reynolds et al., 1990a) show a significant deficit (60%) in the caudate. Also CAT activity was reduced in cortical regions with a deficit of 30% in the hippocampus. However, there was no association of the degree of dementia with CAT activity deficits and indeed the undemented patients had the lowest cortical CAT activity, reaching significance in the temporal cortex (Table 5.2). One possible explanation is that this reduction could be age-related, as the undemented group had a greater mean age and this would predict reduced CAT activity (Rossor et al., 1984). The cortical CAT deficits in Alzheimer's and Parkinson's dementia are much greater than the small reduction seen in HD. Furthermore, cortical concentrations of 5HT and NA, neurotransmitters involved in AD, were found not to be significantly changed in HD dementia (results not shown). Therefore it would appear that HD dementia does not share a similar profile of cortical losses

seen in other dementing diseases; losses which have been assumed to be connected with the production of symptoms of dementia.

Amino acid changes in HD were investigated to assess any relationship with dementia. It was found that cortical and hippocampal GABA concentrations showed no effect between undemented and severely demented patients, contrasting with deficits reported to occur in AD. However, a significant loss of GABA in the caudate (but not the putamen) was observed in the severely demented group (Table 5.3). This is consistent with the hypothesis that HD dementia is subcortical in origin and related to neuronal losses in the caudate. Glutamate concentrations showed similar effects. In HD dementia the hippocampus, frontal cortex and putamen showed minor deficits (10-17%), whereas the caudate was found to have a significant decrease of 42%, compared with undemented patients (Table 5.4).

It is unlikely that these changes are artefactual reflections of other differences between the two HD subgroups, particularly since the results are so regionally specific. There is little difference between the duration of disease in each group indicating that in general the disease process is not more advanced in the demented patients. The effect, if any of the lower age of this group would be to increase GABA concentrations, not to lower them, as is apparent here. Post-mortem delay, agonal state and gender are other factors that are important to consider as potential sources of artefact in the investigation of human autopsy tissue: however, they are unlikely to be responsible for any substantial differences (see section 3.2) in amino acid concentrations in this series.

Table 5.2

Choline acetyltransferase activity in the brain in Huntington's disease: dementia

Values are means ± s.d. in umol/h/g tissue. Nos. of cases in parentheses.
*p < 0.05, ***p < 0.001 versus controls
**p < 0.01 versus severely demented cases.

	Hippocampus	Temporal cortex	Frontal cortex	Caudate
Controls	1.04 ± 0.42	0.38 ± 0.06	0.34 ± 0.09	5.51 ± 2.03
Huntington's disease	0.72 ± 0.42***	0.34 ± 0.11	0.30 ± 0.11*	2.21 ± 1.83***
Not demented (4-5)	0.75 ± 0.37	0.26 ± 0.05**	0.26 ± 0.05	-
Severely demented (10-12)	0.63 ± 0.30	0.41 ± 0.10	0.31 ± 0.10	-

Table 5.3

GABA concentrations in the brain in Huntington's disease: dementia

Values are means ± s.d. in ug/g tissue. Nos. of cases in parentheses.
*p < 0.05, **p < 0.01, ***p < 0.001 versus controls
+p < 0.001 versus severely demented cases.

	Caudate	Putamen	Hippocampus	Frontal cortex
Controls	314 ± 90	461 ± 62	196 ± 55	196 ± 37
Huntington's disease	100 ± 40***	165 ± 52***	140 ± 43***	140 ± 37***
Not demented (4-5)	146 ± 26+	186 ± 49	133 ± 62	131 ± 44
Severely demented (10-12)	81 ± 23	149 ± 14	139 ± 24	138 ± 39

Table 5.4

Glutamate concentrations in the brain in Huntington's disease: dementia

Values are means ± s.d. in ug/g tissue. Nos. of cases in parentheses.
*p < 0.05, **p < 0.01, ***p < 0.001 versus controls
†p < 0.05 versus severely demented cases.

	Caudate	Putamen	Hippocampus	Frontal cortex
Controls	1694 ± 219	2160 ± 272	1232 ± 166	1507 ± 272
Huntington's disease	1127 ± 384***	1638 ± 397	1082 ± 298**	1366 ± 407*
Not demented (4-5)	1628 ± 559†	1842 ± 527	1228 ± 381	1436 ± 736
Severely demented (10-12)	942 ± 301	1535 ± 287	1104 ± 280	1267 ± 430

While this is the first report providing direct neurochemical evidence implicating caudate nucleus in the dementia of HD, there have been other studies indicating caudate nucleus and not the cerebral cortex to be the site of the lesion involved in this cognitive deficit. Several computerised tomography studies have shown cognitive deficits in HD to be correlated with various measures of caudate, but not cortical atrophy (Bamford et al., 1989; Starkstein et al., 1988). Positron emission tomography, used to measure regional metabolic activity, has also demonstrated that there is no hypometabolism in the neocortex in HD, contrary to that in AD and that a decrease in the energy metabolism of the caudate is related to impaired cognitive function (Berent et al., 1988).

The innervation of the caudate nucleus by the cortex is glutamatergic and thus the decrease in caudate glutamate that we observe here is consistent with a loss of this cortical output pathway. As Weinberger et al. (1988) have shown, HD does not show the expected deficit in cerebral blood flow associated with a measurable deterioration of a function mediated by intrinsic frontal cortical systems. Thus the additional neuronal damage of the caudate nucleus that is reflected by further amino acid losses in dementing HD patients can lead to this disruption of cognitive behaviour that is essentially cortical in origin.

Chapter 6

Quinolinic acid

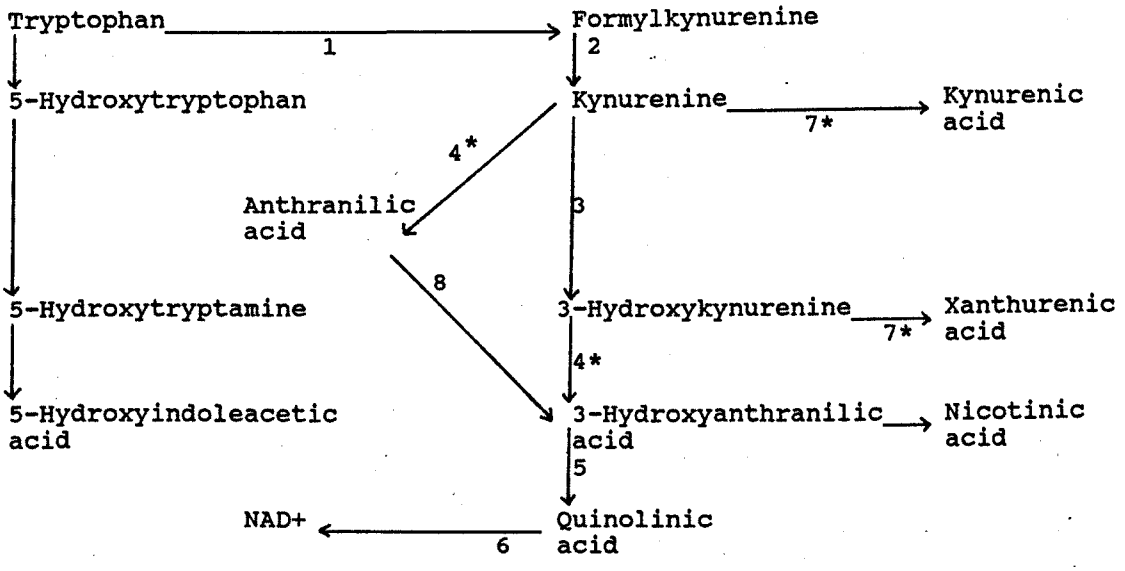
6.1 Metabolism of quinolinic acid

Quinolinic acid (QA; 2,3 pyridine dicarboxylic acid) is an intermediate of the kynurenine pathway (Fig. 6.1), which results in the formation of nicotinamide adenine dinucleotide (NAD) via the metabolism of tryptophan, in the periphery. The kynurenine pathway is the major route for tryptophan metabolism, compared with that producing 5-hydroxytryptamine (5HT) (During et al., 1989a; Gal and Sherman, 1980; Stone and Connick, 1985). Although components of the kynurenine pathway have been identified in the brain, it has been suggested that they may not be metabolised locally, but originate from precursors in the periphery, and that QA, which cannot cross the blood-brain barrier (Foster et al., 1984a) is being synthesized from such a precursor (Speciale et al., 1989b).

QA is synthesized from 3-hydroxyanthranilic acid (3HA) (via spontaneous metabolism of α-amino-β-carboxymuconic-δ-semialdehyde (ACMS)) and metabolized to nicotinic acid mononucleotide (NAMN).

Figure 6.1

Tryptophan metabolism



1= Tryptophan 2,3-dioxygenase (periphery)
Indoleamine 2,3-dioxygenase (brain)

2= Kynurenine formylase

3= Kynurenine hydroxylase

4= Kynureninase

5= 3-Hydroxyanthranilic acid oxygenase

6= Quinolinic acid phosphoribosyltransferase

7= Kynurenine transaminase

8= Anthranilate hydroxylase

*= Vitamin B6 dependent

In the rat brain, the synthetic enzyme, 3-hydroxyanthranilic acid oxygenase (3HAO) and the catabolic enzyme, quinolinic acid phosphoribosyltransferase (QPRT) have been localized to glia. They have a heterogeneous distribution; 3HAO has been demonstrated immunohistochemically to be almost exclusively astrocytic with the caudate, septum, nucleus accumbens, neocortex and hippocampus showing the highest density of 3HAO cells; whereas QPRT was found to be localized in a variety of glial cells with the olfactory nerve, medial septum and diagonal band of Broca showing the highest density of QPRT cells (Kohler et al., 1988). QA has been identified in the rat brain with greater concentrations in the cortex than in the striatum (Wolfensberger et al., 1983). Schwarcz et al. (1988) showed that human brain 3HAO activity is 2-3 times higher than in rat brain, whereas QPRT activity is comparable in both. Their evidence also suggested that 3HAO too was glial-based in the human brain, with the substantia nigra (s.nigra) having the highest 3HAO activity, followed by hypothalamus, globus pallidus, medulla, striatum, cerebellum, thalamus, cortex and hippocampus. Activity of QPRT in the human brain also showed regional variations in order of decreasing magnitude: caudate, s.nigra, thalamus, hypothalamus, cortex, hippocampus, cerebellum and putamen. The cellular localization of QPRT in human brain is indefinite, although glia were proposed (Foster et al., 1985).

The metabolism of tryptophan is subject to many controlling factors. The activity of tryptophan pyrrolase (a metabolic enzyme of tryptophan) is important in relation to the activity of the kynurenine pathway and thus the metabolism of QA. The activity of this enzyme can be affected by corticosteroids or tryptophan loading.

Acute intra-peritoneal (i.p.) injection of hydrocortisone increases activity in rat liver, but not rat brain, whereas Connick and Stone (1988) demonstrated that chronic i.p. administration caused reductions in QA and 5HT metabolism in the rat brain. They suggested that brain tryptophan metabolism was reduced as a result of the increase in liver tryptophan metabolism. Systemic tryptophan loading has been shown to increase tryptophan pyrrolase activity (Stone and Connick, 1985) as well as increasing concentrations of QA, 5HT and tryptophan in the blood, brain and brain extracellular fluid (Heyes and Markey, 1988b; Moroni et al., 1984a; During et al., 1989a). The effect on QA is the greatest, indicating a stronger relationship between QA and its precursor. I.p. tryptophan administration produced similar effects, which were dose-dependent, with QA showing over 200-fold increase at the highest dose (During et al., 1989a).

There is some contrary evidence regarding the in vivo production of brain extracellular QA after exposure to tryptophan, as well as other precursors. Speciale et al. (1989b) found that tissue perfusion of tryptophan or kynurenine did not raise extracellular QA above the limit of sensitivity of their assay, whereas 3HA produced a dose-dependent increase in extracellular QA, until steady state, of up to several hundred-fold compared to basal levels. They suggest that this is the first evidence of a functional pathway in vivo from extracellular 3HA to QA in the rat brain. The data above regarding the effect of systemic tryptophan loading, as compared with the in vivo perfusion, imply that a kynurenine metabolite is produced in the periphery and is metabolized locally to produce QA, crossing the blood-brain barrier which is impenetrable to QA.

Systemic kynurenine also increases rat brain concentrations of QA (Speciale et al., 1989b), but as kynurenine is freely able to cross the blood-brain barrier (Gal and Sherman, 1978) this does not provide evidence for a functional kynurenine pathway in the brain. Of interest are the data from Moroni et al. (1985) which shows that rats fed a tryptophan-free diet had doubled QA levels in the cortex, compared with controls, whereas 5HT and 5-hydroxyindoleacetic acid (5HIAA) concentrations were decreased. These animals also displayed the symptoms of pellagra. This is contrary to the consistent relationship between systemic tryptophan and brain QA, described earlier. Furthermore, rats treated with p-chlorophenylalanine (a drug that decreases concentrations of tryptophan and 5HT in the brain of rats, by inhibiting the enzyme tryptophan hydroxylase) also had reduced concentrations of cortical QA (Moroni et al., 1984a).

Neuronal damage is a further factor affecting QA metabolism. Neurodegeneration following excitotoxic administration has been shown to be associated with increased activity of 3HAO and to a lesser degree, QPRT. This was demonstrated for a variety of neurotoxins including kainic acid (Speciale et al., 1988), ibotenic acid (Schwarcz et al., 1989) and QA itself (Foster et al., 1985). Furthermore, Speciale et al. (1989b) reported tissue perfusion with 3HA in ibotenate-lesioned tissue caused an 'accelerated surge in extracellular QA to the same maximal levels as controls'. These findings have been suggested to reflect the relative gliosis resulting from the neuronal losses, as QA metabolism (as discussed above), is thought to be glial-based. However, Speciale et al. (1987) provided evidence to suggest new synthesis of both 3HAO and QPRT, albeit to a

lesser extent. They hypothesized that a small neuronal loss may result in increased production of QA, which in turn would cause yet more neuronal losses, thus forming a feed-forward system.

Other stimuli have also been demonstrated to change QA metabolism. Exposure to endotoxin (lipopolysaccharide of bacterial origin) or pokeweed mitogen (plant antigen) has been demonstrated to result in increased QA concentrations in the mouse cortex (Heyes et al., 1988) and systemic endotoxin also increases concentrations of tryptophan, 5HIAA and 3-hydroxykynurenine (3HK) in the mouse cortex (Heyes et al., 1989a). They suggested that increased systemic tryptophan metabolism following infection or endotoxin (resulting from increased tryptophan release from skeletal muscle) also extended to the brain in mice. This increased tryptophan metabolism was also potentiated when tryptophan was administered. Tryptophan competes with large neutral amino acids for uptake into the brain. An increased ratio of tryptophan to large neutral amino acids, could cause an increase in brain tryptophan metabolism, as more tryptophan is taken up into the brain. Thus, there would be less peripheral tryptophan (e.g. reduced serum concentrations). A further factor affecting QA is probenecid. QA is a substrate of the probenecid-sensitive organic acid transport system (Connick et al., 1988) and probenecid treated rats had twofold amount of QA in the cortex, as compared to controls (Moroni et al., 1986b).

6.2 Neurotoxins

Certain excitatory amino acids,'excitotoxins', can cause neurodegeneration by excessive excitation when administered to the brain. The potency of these compounds appears to be strongly related to their neuroexcitatory capacity (Schwarcz et al., 1984b). Characteristic lesions are formed which are 'axon-sparing'; that is, extrinsic axons and non-neuronal cells are spared and intrinsic neurones are lost (Olney, 1971). It has been suggested that an excess of endogenous neurotoxins may be responsible for the neurodegeneration found in diseases such as epilepsy and Huntington's disease (HD) (Feldblum et al., 1979; Coyle and Schwarcz, 1976). Glutamate is one such compound, but large amounts are needed in the mature rat to produce neurotoxic damage, unless a reduction in high-affinity uptake sites occurs (Manango and Schwarcz, 1983; Köhler and Schwarcz, 1981). Of more interest are two exogeneous compounds, kainate and ibotenate, which structurally resemble glutamate. Injection of these compounds into the striatum or hippocampus produced axon-sparing lesions that provided the early animal models for HD and epilepsy (Coyle and Schwarcz, 1976; Nadler, 1981), although subsequently, QA was demonstrated to provide a better animal model of HD (Schwarcz et al., 1983; Beal et al., 1986).

6.2.1 Selectivity of QA as an animal model for HD

There are certain similarities between the three HD models. Intra-striatal injection resulted in lesions that morphologically and neurochemically resembled many of the cell losses and changes seen in the HD striatum. Thus, losses of medium spiny neurones occurred resulting in reduced markers of their neurotransmitters, γ-aminobutyric acid (GABA),

substance P and acetylcholine. There was sparing of large or medium aspiny neurones and the survival of the striatal afferents was reflected by unchanged or increased levels of dopamine, vasopressin and 5HT markers. However, there was a major difference between the three models, that involved a selective sparing of somatostatin (SS)/ neuropeptide Y (NPY)/ NADPH-d containing neurones, which are not lost in the HD striatum; only the QA model spared these (Beal et al., 1986). Furthermore there were other anomalies regarding the kainate and ibotenate animal models of HD. Kainate administration resulted in lesions away from the site of injection and produced strong convulsive effects; ibotenate was found to affect immature rat neurones, whereas HD does not usually develop until middle age (Schwarcz et al., 1983). Thus, a functional excess of the endogenous neurotoxin QA may have an aetiological role in HD. However, there is also substantial evidence disputing the selectivity of the QA model. A long inconclusive debate has continued between Davies/ Roberts and Beal et al. regarding the preservation of the SS/ NPY/ NADPH-d neurones. Davies and Roberts (1987/1988) used immunocytochemical and enzyme histochemical methods to measure numbers of these neurones and found no selective sparing, and suggested that the increased peptides measured by Beal et al., using radioimmunoassay, may reflect their presence in striatal afferents. Beal et al. (1987/1988b) defended and repeated their results and suggested that the Davies evidence was taken from measurements of the absolute core of the lesion, where there is total neuronal loss; whereas they should have used an outer region exhibiting 50% loss, where selective increases occur. Furthermore, they argued that striatal afferents are also spared in kainate lesions, but there is no selective sparing of the SS/ NPY/ NADPH-d neurones.

There is evidence supporting the selective QA lesion (Koh et al., 1986; Nemeroff, personal communication to Beal, 1987) as well as negating it (Boegman et al., 1987; Davies and Roberts, 1988). Both groups agree regarding a further selective sparing: the cholinergic neurones, especially at the core of the lesion (Davies and Roberts, 1988; Boegman et al., 1987; Beal et al., 1988b). This is concordant with preserved groups of cholinergic neurones in HD (Ferrante et al., 1987b), but this effect occurs with kainate and ibotenate also. If indeed the SS/ NPY/ NADPH-d neurones are preserved, there has been speculation as to why. Koh et al.(1986) suggested that these cells could have a lack of receptors through which the neurotoxic effects of QA are mediated, the NMDA receptor. Alternatively, Beal et al.(1986) suggested that maybe these cells were metabolizing QA to NADP or NADPH, as quinones are detoxified by NADPH-d (Chesis et al., 1984). The relative sparing of the nucleus accumbens in HD could also be explained, as the nucleus accumbens has 2-3 fold more of these neurones than other areas, and thus is protected. A further hypothesis is that a particular property related to cells, not having spines, is protective (Ferrante et al., 1987b). The neurotoxic effects of QA are not only defined by which neurones are lost, as there is also a regional variation. Schwarcz and Kohler (1983) demonstrated that an infusion of QA was more neurotoxic in the rat striatum, pallidum, hippocampus and venteromedial pallidum (equivalent to the human basal nucleus). More resistant were the cerebellum (especially the Purkinje cells), s.nigra, amygdala, medial septum and hypothalamus. It can be observed that, in general, the more susceptible regions are those that show neuronal degeneration in HD (Ferrante et al., 1987b). Another important feature of QA neurotoxicity is

that neonate rats are resistant, unlike the adult animals (Schwarcz et al., 1984b); this being relevant because HD usually begins in middle age.

6.2.2 Characteristics of QA as a neurotoxin

A point of consideration is the concentrations of QA necessary to produce neurotoxic damage, especially with relevance to the hypothesis that increased QA may have an aetiological role in HD. In the rat QA intra-striatal injections ranging from 12-600 nmole were shown to be neurotoxic, although to varying degrees (Schwarcz et al., 1983) and from in-vitro tissue culture studies, 10 μM QA was neurotoxic (During et al., 1989a). Using their intra-cerebral microdialysis technique, During estimated levels of QA in extracellular fluid of rat striatum to be greater than 14 μM, following a systemic tryptophan load, and are thus potentially neurotoxic. Therefore, tryptophan or any of the other factors affecting QA metabolism (see section 6.1) may potentiate otherwise normal levels of QA, to produce neurotoxic damage. McGeer and Singh (1984) also reported that endogenous QA concentrations in the rat brain are neurotoxic. Recently, Whetsell and Schwarcz (1989) provided evidence that much lower concentrations of QA are possible for chronic neurotoxic damage to occur (which is more relevant to HD as it is an insidious disease). They demonstrated that 100 nM QA, when administered chronically (more than 7 weeks), produced neurotoxic damage to rat cortico-striatal cells in culture. Caudate cells, without cortical cells were unaffected by the same chronic QA concentrations. Thus, they suggested that chronic exposure to only slightly hyperphysiological levels of QA in vivo (> 1 μM) may result in neurotoxic cell losses. A further point made was that a combination of cortex

and striatum was necessary for neurotoxicity. This concurs with studies suggesting an intact cortico-striatal pathway is a prerequisite for QA neurotoxicity in the rat striatum. This may also explain why neonates are resistant to QA neurotoxicity, as they have not developed these afferents to the striatum (Foster et al., 1983). Kim and Choi (1987) also investigated the effects of time using cortical cultures: with short exposure (20 min), QA was only weakly neurotoxic; whereas 4 day exposure multiplies its effects by an order of magnitude. They hypothesised that nanomolar concentrations producing neuronal degeneration in vivo, may reflect the lack of uptake of extracellular QA, resulting in high local concentrations.

A consequence of the presence of QA is the competitive inhibition of monoamine oxidase (MAO)-B in human brain synaptosomes, as demonstrated by Naoi et al. (1987), with MAO-A not being affected. 3HA and xanthurenic acid also inhibited MAO-B, but non-competitively. Thus, QA may be an endogenous modulator of monoamines in the brain.

6.2.3 Other neurotoxic compounds (adapted from Schwarcz et al., 1984b)

Exogenous:	Endogenous:
kainate	glutamate
ibotenate	homocysteic acid (HCA)
N-methyl D-aspartate (NMDA)	pyroglutamate (PGA)
phthalic acid	nicotinic acid
quisqualate	tetrahydrofolate
trans 2-3 PDA	aspartate
cis 2-3 PDA	cysteic acid
AP3	cysteine sulphinate
AP4	homocysteine -sulphinate
	3HK
	picolinic acid
	cysteine

(PDA = piperidine dicarboxylic acid; AP = 2-amino-phosphonic acid)

None of the above compounds have proved to produce as good an animal model for HD as QA (Schwarcz et al., 1984b; Beal et al., 1986; Watkins and Olverman, 1987), except PGA and HCA which have provoked some interest. PGA was postulated to be aetiologically involved in HD, but it produces minimal neurodegeneration even using high concentrations (Rieke et al., 1984). Intrastriatal injection of PGA in the rat produced dyskinesias but reduced striatal PGA was found in HD, accompanied by a peripheral increase (plasma), thus negating any hypothetical aetiological role (Uhlhaas and Lange, 1988). However, the latest study of Rieke et al. (1989) provides evidence that chronic intrastriatal PGA produces neuronal losses similar to those found

in HD. More recently Beal et al.(1990b) suggested that HCA could be pathologically involved in HD. HCA lesions in the rat striatum produced selective cell losses identical to those induced by QA, and which could be blocked in similar means. However in vivo, HCA has a relatively low potency (2000-4000 nmol produced 40% losses of GABA); whereas in vitro it was shown to be much more potent and this was said to reflect active re-uptake sites in vivo. New evidence from Perry and Hansen (1990) demonstrated that HCA is probably not abnormally increased in the HD striatum; also that patients with homocystinuria (with increased HCA) do not have the symptoms of HD. Thus evidence does not support the aetiological hypothesis of HCA in HD. An earlier study of Perry et al. (1987) reported that an unidentified neurotoxin caused GABAergic neuronal losses in vitro, when cultured with serum from drug-free HD patients.

6.2.4 Behavioural effects of QA

The animal models of QA were compared with regard to their similarity to the symptomology of HD. Intrastriatal injection of either kainate and ibotenate produced tonic-clonic movements of the contra-lateral forelimb in rats (Coyle and Schwarcz, 1976). Schwarcz and Kohler (1983) demonstrated that similar locomotor changes occurred when QA was injected unilaterally (> 150 nmole) lasting 4-6 hours. This was accompanied by episodic barrel-like rotations and was dose-dependent. Only very large (> 500 nmole) intrahippocampal injections produced convulsions, turning, running fits and jumping. Sanberg and Fibiger (1989) assessed the behavioural abnormalities induced in rats by bilateral intrastriatal QA (75, 150, 225, 300 nmol). They compared these changes to those reported to occur

with kainate and ibotenate: learning difficulties, persistent nocturnal locomotor hyperactivity, altered feeding and drinking mechanisms and potentiated locomotion when aroused. These were said to be similar to HD symptoms and although chorea was not seen, shorter paw-ground contact with longer swing time was postulated to be analagous to HD patients movements. QA lesions produced similar abnormalities to kainate and ibotenate, but these were dose-dependent. A dose of 75 nmol QA produced no locomotor impairment, and the 300 dose was fatal, as was the 225 dose to one third of its group. The 150 nmol and 225 nmol doses produced similar effects to a 3 nmol dose kainate. It was suggested that intermediate doses of QA (150-225 nmol) may be more useful to study. Sanberg and Fibiger (1989) also reported dysfunctions in metabolism, such as weight loss or feeding. QA doses of 150 nmol and 225 nmol induced an initial weight loss, also seen with kainate lesioning. This was likened to the cachexia seen in HD, with increased appetite. This weight loss has been postulated to reflect increased calories used in the dyskinesia, but this hypothesis has not been substantiated, and indeed the weight loss could reflect a metabolic dysfunction such as altered fat metabolism. Further evidence regarding behavioral deficits comes from studies involving the nucleus basalis. Unilateral injection of (120 nmol) QA into this region was shown to produce decreased cortical choline acetyltransferase activity together with memory impairment (radial maze test) (Beninger et al., 1986).

6.2.5 QA as an animal model for temporal lobe epilepsy

Seizures have been induced in rats by the administration of systemic, intra-ventricular or intra-hippocampal excitatory amnio acids (including ibotenate, kainate or QA) (Schwarcz and Kohler, 1983). Initially kainate was thought to provide the best model as it selectively damaged the pyramidal cells of the hippocampus, which are affected in temporal lobe epilepsy. The effects of kainate lesions were reduced by prior hippocampal deafferentation which has a similar action to temporal lobotomy used in extreme cases of the disease. Ibotenate lesions showed neither of these characteristics; furthermore, kainate produced neuronal degeneration at sites distant to the hippocampus, unlike epilepsy. Thus, a convincing animal model was not evident using kainate or ibotenate (Schwarcz et al., 1984b). Evidence from studies using QA suggest that its excitotoxic properties provide a better animal model for temporal lobe epilepsy. Intra-hippocampal injections of > 500 nmole QA resulted in generalised convulsions and 30 nmole QA produced the selective losses of pyramidal cells, but without lesions distant from the injection site (Schwarcz and Kohler, 1983).

6.2.6 Neurological disorders possibly associated with excitotoxins

Other clinical states postulated to involve excitotoxic damage include glutaric aciduria-I (GA-I), stroke, motor neurone disease, hypoglycaemia, febrile convulsions and Alzheimer's disease. QA may be involved in the recessive, neurodegenerative, dyskinetic disorder, GA-I (Heyes, 1987), which bears

some resemblance to HD, both clinically and neuropathologically (Goodman et al., 1977). Pathological changes include atrophy of the putamen, caudate and cortex, as in HD, and clinical symptoms may include dystonia, chorea, athetosis and speech dysarthia. The precursor of QA, ACMS, also forms an alternative pathway, to form glutaric and glutaconic acids (lysine metabolites). As there is a deficiency of glutaryl-CoA dehydrogenase and glutaconyl-CoA decarboxylase, there is increased excretion of glutaconic, glutaric and 3-hydroxyglutaric acids and glutarylglycine. This enzymatic deficiency would be expected to result in increased metabolism of ACMS to QA, thus it has been hypothesized that increased brain QA may be having aetiological neurotoxic effects (Heyes, 1987). Brain QA has not been determined, however a possible animal model can be used, where ACMS decarboxylase is blocked by pyrazinamide in rats, thus causing a flux through the QA pathway. Furthermore, systemic tryptophan-loading, which normally results in increased cortical QA, has twice the effect with pyrazinamide pretreatment (Heyes, 1987). Further support for an aetiological role for QA in GA-I is provided by Heyes et al. (1989a). Cortical QA and 3HK concentrations are increased following infection, in the mouse, which was suggested to be consistent with neurological deterioration in GA-I occurring at times of infection. A possibly related disorder, Rett's syndrome, has also been linked with disturbance of glutaric acid metabolism (Chaves-Carballo, 1987). Evidence suggests that hypoxic-ischaemic neuronal damage may be mediated via the same receptor as QA and prevented by the same antagonists (Simon et al., 1984; Germano et al., 1987). Furthermore, the same pattern of cell loss produced by QA is seen in the hypoxic-ischaemic animal model, induced by carotid ligation (Ferriero

et al., 1988). Similar mechanisms have been suggested to be involved in hypoglycaemia-induced neuronal damage (Simon et al., 1986; Wieloch et al., 1985) and QA has also been implicated in the production of febrile convulsions (Legidio et al., 1988). A possible role for QA in Alzheimer's disease (AD) has also been postulated in view of the sensitivity of the nucleus basalis to neurotoxic damage; these neurones are known to be lost in AD. Injection of QA into this area in rats produced neuronal losses accompanied by memory deficits (Beninger et al., 1986).

6.3 Neuropharmacological actions of QA

Evidence suggests that there are two types of excitatory amino acid receptors: NMDA (N-methyl-D-aspartate) and non-NMDA. Kainate and quisqualate are thought to act through non-NMDA receptors and QA actions have been suggested to be mediated via the NMDA receptor (Watkins and Evans, 1981; Ganong and Cotman, 1986).

6.3.1 NMDA receptors

NMDA receptors are distributed throughout the brain, with the CA1 region of the hippocampus having the greatest density in both rat and man, followed by specific layers of the cortex, basal ganglia, septum and amygdala (Monaghan and Cotman, 1985). They are structurally complex, with many potential sites of action and exist in two conformational states: resting and activated. Several binding sites have been demonstrated to co-localize with NMDA receptors, one example being the strychnine-insensitive high-affinity glycine site. Glycine has been shown to increase the frequency of NMDA receptor channel opening possibly involving

allosteric regulation via a separate site (Foster and Kemp, 1989), thus allowing increased binding to the site within the channel. Kleckner and Dingledine (1988) suggested that glycine and glutamate acted as co-agonists as both were vital for NMDA receptor response, whilst Mayer et al. (1989) demonstrated that glycine induced faster recovery from desensitization. Foster and Kemp (1989) suggested that the action of glycine may be a combination of both effects. Furthermore, D-serine produces similar modulatory effects to glycine (Wood et al., 1989). Procter et al. (1989) demonstrated impaired coupling between the glycine site with the agonist site and a site within the channel, in the cortex in AD. They postulated that the physiological function of glycine may be relevant in this and other neurodegenerative disorders. Consequently glycine antagonists, at this site, such as HA-966 or kynurenic acid (KA), may provide useful tools for the manipulation of NMDA effects in these diseases. Another site co-localized with NMDA receptors is the phencyclidine (PCP) receptor. PCP (as well as KA, MK801, SKF10047, ketamine, α-aminoadipate, tetrahydroaminoacridine) act as non-competitive antagonists within the open channel of the NMDA complex (Watkins and Olverman, 1987; Kemp et al., 1987; Davenport et al., 1988). A sigma-opiate receptor (now called sigma receptor) was thought to be associated with the PCP site and thus the NMDA receptor. However recent evidence suggests that they are anatomically separate and are defined by non-dopaminergic haloperidol, DTG and +3PPP binding. They have a different localization to NMDA receptors, but agonists for sigma sites have effects at NMDA sites (Itzhak and Stein, 1990; Weissman et al., 1988). Divalent cations (e.g. magnesium, calcium and zinc) are a further group of non-competitive antagonists of NMDA receptors. They are

thought to act by physically blocking the open channel, which may also interact with PCP site antagonists (Kemp et al., 1987; Peters et al., 1987). There are competitive antagonists that act at the agonist site of the NMDA receptor, preventing receptor activation. These include D-AP5, D-AP7 and CGP39653. A further site is a polyamine site (e.g. spermidine). Spermidine has been shown to enhance glutamate at a site distinct from NMDA or glycine receptors (Ransom and Stec, 1988). Ethanol has also been shown to act as an antagonist and Hoffman et al. (1989) suggested that it acts on the co-agonist (glycine site) rather than the PCP site. Other antagonists whose mechanisms are unknown, include glutathione and ascorbic acid (Majewska et al., 1989).

Young et al. (1988) determined NMDA, PCP and quisqualate binding in HD brain and controls. In the putamen NMDA receptor binding was reduced by 93% as compared to 55-67% losses for the other receptors. There were no changes in the cortex. The lack of correlation between PCP and NMDA binding was unexpected and unexplained. They suggested that the loss of NMDA receptors in the HD putamen could be due to neurotoxic neurodegeneration, or a primary defect in striatal NMDA receptors, or susceptibility due to defective energy metabolism.

6.3.2 QA as an NMDA agonist

There is much evidence supporting the theory that QA acts via NMDA receptors. Iontophoretical or peripheral (i.p.) application of QA caused excitation of the rat neocortex which could be blocked by specific NMDA antagonists (e.g. 2-APH) (Stone and Perkins, 1981; Perkins and Stone, 1983a, 1983b). These effects of QA have been demonstrated

to be regionally specific; thus the striatum, hippocampus and neocortex showed the greatest response and cerebellum and spinal cord, the least. Furthermore there was a difference between cell-sensitivity to glutamate and QA, which could be predicted as glutamate does not act solely on NMDA receptors. However NMDA itself produced a different profile to QA. Perkins and Stone (1983c) hypothesized that there were sub-types of NMDA receptors (1 and 2 sites), with NMDA1 only acted on by NMDA, and NMDA2 activated by both NMDA and QA. A consistent finding is the differential release of acetylcholine induced by QA and NMDA (Lehman, 1983). Garthwaite and Garthwaite (1987) showed that both the excitotoxic and neurotoxic properties of QA and NMDA were related to their relative affinities for NMDA receptors. Studies have shown young rats to be more sensitive to QA, having an excitotoxic threshold dose of 50 compared to 10000 (mg/kg i.p.) in adult rats (Czuczwar and Meldrum, 1982). It has been reported that doses of QA that induce neurotoxicity may not be convulsant, thus differential blockage of selective effects can be achieved (Schwarcz et al., 1984a; Vezzani et al., 1989).

NMDA-mediated neurotoxicity is composed of two stages, the first, an initial acute sodium entry, accompanied by entry of water resulting in swelling and eventual cell lysis. The later stage involves calcium influx into the cell which triggers cell degeneration. The consequences of this include such effects as activation of proteases and phospholipases, increased formation of free radicals, swelling of the mitochondria and golgi and cytoplasmic damage and aggregation of the nucleus (Choi, 1990; Garthwaite, 1990). Swelling is not a prerequisite for neurotoxicity; Rothman and Olney

(1987) hypothesized that neurotoxicity is related to calcium influx through agonist-gated channels (especially NMDA), causing mitochondrial dysfunction, lipase activation and increased NMDA receptor activity. Evidence supporting these theories comes from studies made by Kim and Choi (1987). Using cortical slices, they showed that QA could induce an acute, sodium-dependent excitotoxic cell swelling, and a late calcium-dependent neurodegenerative stage. However, Vezzani et al. (1988) studied the role of calcium on the neurotoxicity and convulsant properties of QA, in the rat hippocampus. Calcium entry into the cell often precedes the onset of seizures and is also associated with the activation of NMDA receptors. Their results indicated that a decrease in extracellular calcium, and thus an inward calcium current, was associated with QA-induced seizures. Treatment with drugs that block seizures (carbamazepine, flunarizine) were shown to prevent the extracellular calcium decrease. Contrary to this, the neurotoxic effects of QA were unaffected by calcium concentrations. Both the neurotoxic and excitatory effects were blocked by 2APH, demonstrating that NMDA receptors are involved although through different mechanisms. Thus they postulated that calcium influx may be a causative factor in temporal lobe epilepsy induced by excitatory amino acids.

Schwarcz et al. (1984b) postulated an extra presynaptic role for QA suggesting that it might act at a presynaptic receptor causing the release of endogenous transmitter (e.g. glutamate), which then acts on the post-synaptic NMDA. This theory is consistent with reports that afferent projections are necessary for QA neurotoxicity. Connick and Stone (1989) provides further support for a presynaptic site. QA stimulates excitatory amino

acid release and purines suppress it, and as co-injection of a purine agonist (phenylisopropyladenosine, PIA) protects against QA neurotoxicity, this may explain the mechanism of protection. PIA is also a vasodilator and systemic administration paradoxically potentiated QA. They explained this as having induced hypotension, which interacted with cells already damaged by QA resulting in increased neuronal degeneration.

6.3.3 Pharmacological antagonism of QA

Specific NMDA antagonists can attenuate or block the effects of QA. 2APH and 5APH were the first competitive antagonists demonstrated to prevent QA induced seizure and neurotoxicity. However these compounds were of limited value as they showed limited uptake into the brain and were inactive when given orally (Schwarcz and Meldrum, 1985). Potentially more promising was the evidence suggesting that another endogenous kynurenine metabolite, KA, blocked neurotoxicity and seizures induced by QA, in striatum and hippocampus of rats (Foster et al., 1984b). They showed morphologically and neurochemically, that co-injection of KA preferentially blocked the effects of QA, when compared with effects of other acidic amino acids (including NMDA). They suggested that KA and QA share the same functional pattern and maybe linked functionally as well as metabolically. Further evidence from Beninger et al.(1986) demonstrates the protective role of KA, in preventing memory and neuronal losses induced by QA in the nucleus basalis. They and others (Foster et al., 1984b) have postulated that a change in the balance between QA and KA may be important in neurodegenerative disease. In particular, that KA may be relatively decreased in HD (Stone and Connick, 1985). A

further effect of KA was shown to be the attenuation of infarct size and locomotor deficits in an animal model of stroke, after pretreatment (Germano et al., 1987).

Systemic administration of the non-competitive antagonist MK801 has been shown to prevent QA-induced neurotoxic damage, and also seizures but at a lower threshold dose suggesting only partial blockade of the NMDA receptor (Kemp et al., 1987; Vezzani et al., 1989). They also reported that alpha-1 adrenoreceptors have been suggested to play a role in the anti-convulsant action of MK801, and QA-induced seizures can be inhibited by their activation. MK801 showed easy uptake into the brain, but at low doses induced motor impairment and it has also been reported to protect against ischaemic neurodegeneration in the gerbil hippocampus (Foster et al., 1987). Studies have also shown systemic ketamine to provide incomplete protection against QA, blocking excitation only (Lees, 1987), whereas dipicolinic acid (an analog of QA) provided complete protection (Boegman et al., 1986). Systemic pretreatment with GM1 ganglioside was shown by Lombardi et al. (1989) to protect against QA neurotoxicity, however this is not thought to have an action at NMDA receptors and they hypothesized that it may be incorporated into cell membranes, preventing excitoxic neurodegéneration.

6.4 Methods of measuring QA metabolism

6.4.1 Enzymes involved in QA synthesis or metabolism

QA metabolism can be described either by assessing the synthetic and metabolic enzyme activity, or by measuring QA concentrations. The presence and characterization of 3HAO in rat brain was demonstrated by Foster et al. (1986b) using a

radioenzymatic assay. This involved the production of (^{14} C) QA from (carboxy-^{14} C) 3HAO. Further localization of brain 3HAO was carried out using immunohistochemical techniques using anti-3HAO antibodies raised against purified liver 3HAO (Köhler et al., 1988a). The radioenzymatic assay described above has been applied to brain tissue from patients with HD (Schwarcz et al., 1988a). Results show 3HAO to be significantly increased in HD brains, especially in the putamen and caudate, but in most other areas also. There was no correlation between neuropathology and 3HAO activity. It was suggested that this increase was merely reflecting astrogliosis, although less atrophied areas still showed this increase in 3HAO activity.

QPRT has been purified from rat liver and brain and characterized (Okuno and Schwarcz, 1985). Again a radioenzymatic assay was used in rat and human brain which determined QPRT activity by measuring the formation of (3 H) NAMN from (3 H) QA (Foster et al., 1988). Similar immunohistochemical localization, as described for 3HAO above, was carried out and here too the radioenzymatic assay of QPRT activity was measured in HD brain tissue (Foster et al., 1985). Results demonstrated that the activity of QPRT was not significantly increased in caudate or putamen of HD brain, although there was a 'trend',towards increased values, but only in the caudate. Preliminary data further extended this lack of difference to the s.nigra, hypothalamus, cerebellum and cortex. Kish et al., (1991) recently reported increased cerebellar activity of QPRT (but not 3HAO, and not significantly changed in the cortex) in brain tissue from patients with dominantly inherited olivopontocerebellar atrophy. They suggested that this may be a marker for

increased catabolism to remove QA from sensitive granule cells in this disorder. In brains from epileptics, QPRT activity has been found to be decreased in frontal and temporal cortex, but not in the amygdala or Ammon's horn (Feldblum et al., 1988). The effect of this was hypothesized to create or maintain an epileptic focus.

6.4.2 QA concentrations in the periphery and cerebrospinal fluid

Concentrations of QA were first determined in tissue and body fluids, using assays that were not sensitive to measure brain levels. Early techniques for estimating urinary QA involved a microbiological method (Henderson and Hirsch, 1949), paper chromatography (Jakoby and Bonner, 1951), ion-exchange followed by colorimetric detection (Heeley et al., 1966) or gas-liquid chromatography (Toseland, 1969), thick-layer chromatography (Crawford et al., 1972), high performance liquid chromatography (HPLC) and spectrophotometric detection (Patterson and Brown, 1980). Human urine concentrations of QA/24 hour varied from 25 μmoles (Patterson), 36-78 μmoles (Toseland), and following a tryptophan load of 5 g, increased to 132-816 μmoles (Toseland). Heyes et al. (1985) measured QA concentrations in urine from HD patients and controls. Although there was significantly decreased QA/24 hours in the HD group as compared with controls (31 and 52 μmoles respectively), the difference disappeared when the data was corrected relative to creatinine and urea excretion. Using a radioenzymatic assay, Foster et al. (1986a) measured QA in both human urine and plasma (37 pmol/1 μl, 21 pmol/50 μl). Perry et al. (1987) reported no increased QA concentrations in urine, plasma and CSF of HD patients, which concurred with data from

Schwarcz et al. (1988b) in CSF, also measured by radioenzymatic assay. They showed CSF from HD patients to have not significantly changed concentrations of QA (20 nM) compared with schizophrenics (27 nM) and the control range (20-30 nM). Furthermore, they described 'highly variable' QA concentrations in both diseases. Recent studies were also made involving other neurological conditions. QA concentrations showed a threefold increase in CSF from patients with AIDS and were increased in CSF and plasma in HIV infection (Heyes and Markey, 1989b; Heyes et al., 1990a, 1991a). There was a positive correlation between CSF QA and cognitive dementia and motor dysfunction. After treatment with an anti-AIDS drug, AZT (zidovudine), and anti-microbial therapy, QA CSF concentrations were reduced back to normal in association with neurological improvement. Furthermore in an animal model of AIDS (Heyes et al., 1990b), CSF KA concentrations were increased, but to a lesser degree than QA levels. Acute septicemia was also reported to result in increased CSF QA, KA and kynurenine in animals (Heyes and Lackner, 1990).

6.4.3 QA concentrations in the brain

The first identification of QA in the rat and human brain was made by Wolfensberger et al.(1983) using the ion-exchange method described by Chandler and Gholson (1972), followed by HPLC as used by Patterson and Brown (1980), prior to derivatization and detection using electron impact gas-chromatography/ mass-spectrometry (GC-MS). Concentrations (fmol/mg wet weight) in the human brain were 369 (caudate), 567 (frontal cortex), 576 (cerebellum), which were not found to be significantly different from each other. In rat, concentrations were 739 (striatum), 1584 (frontal

cortex) and 434 (cerebellum), demonstrating a difference between species. Later work by Moroni et al. (1984a) confirmed the presence of QA in rat brain (at similar levels to Wolfensberger) using an electron impact GC-MS method; and also in human cortex (0.5-0.8 nmol/g wet weight) (Moroni et al., 1986a, 1986c). The most recent GC-MS method used to measure QA involved the use of electron-capture negative chemical ionization (CI), which was described as being more specific than the usual electron impact (EI) methods used previously. Furthermore that the use of (^{18} O) QA was a more accurate, more suitable internal standard than 2,4 pyridine dicarboxylic acid used by Wolfensberger and Moroni (Heyes and Markey, 1988a, 1988b). In rat frontal cortex, Heyes demonstrated a range of 20-180 fmol/mg QA, with no regional variability throughout the rest of the brain. This contrasts with the Wolfensberger or Moroni data, which gives a range of 500-2000 fmol/mg. Heyes ascribed this difference to increased specificity and sensitivity. Intra-striatal microdialysis with GC-MS was the most recent method used to monitor extracellular fluid (ECF) levels of QA (Speciale et al., 1989b; During et al., 1989b). Speciale et al. were only able to measure ECF QA levels after loading experiments (tryptophan, 3HA, kynurenine) due to the limit of sensitivity of their method (20 nM), which was based on the radioenzymatic assay of Foster et al.(1986a). Heyes and Markey (1988a, 1988b), using their new improved CI GC-MS method demonstrated ECF QA levels of 5.5 nM, which rose after loading with tryptophan and QA. The effects of loading were described previously.

6.4.4 Clinical measures of QA concentrations in the brain

Moroni et al. (1984b) examined the distribution of QA in the rabbit and guinea pig brain; QA was shown to be similarly distributed in all the mammals. An increase in QA with age was demonstrated, this reaching almost neurotoxic levels at 30 months. This was explained to be partially due to the decrease in brain extracellular water, occurring during maturation, as QA was expressed /g wet weight. A further conclusion reported was that newborn rats cortex did not possess the capacity to produce QA, further illustrated by the lack of effect tryptophan loading has in the brain of newborn rats. In the 30 month rats, QA showed a particularly large variability, which had previously been reported by Wolfensberger et al. (1983). A further study by Moroni et al. (1986c) assessed QA concentrations in the cortex of patients with AD and age-matched controls, to examine the hypothesis that increased QA may cause neuronal degeneration in AD. Their results demonstrated that QA concentrations in three areas of the cortex, showed no difference between AD and controls, thus providing evidence against the hypothesis. It was suggested that it may still be of relevance to measure QA in other regions, where there are specific cell deficits in AD, such as the hippocampus and nucleus basalis (Ball et al., 1985). The lack of change in the brain in AD is important, as if QA is increased in the brain in HD, these results provide evidence that the effect is not merely reflecting tissue atrophy. A further finding was that there was no relationship between cortical QA and the severity of AD, or with age (in contrast with the effect of age on QA concentrations in rats). The only studies of brain QA that provided positive results, were experimental

models of hepatic encephalopathy in the rat (Moroni et al., 1986b) and patients with hepatic failure (Moroni et al., 1986a). Rats bearing a portocaval anastomosis (PA), so that the blood bypasses the liver, were demonstrated to have increased QA concentrations in the cortex and cerebellum (75% and 125% respectively, compared with controls). 5HT was increased only in the brainstem, but 5HIAA was increased throughout the brain, reflecting increased metabolism of 5HT. Probenecid (a blocker of acid transport systems) inhibits QA transport mechanism and caused a twofold increase in control cortical QA. However in PA animals, probenecid surprisingly failed to cause this increase, although it did result in even greater 5HT metabolism. An aetiological role for tryptophan was suggested by Moroni, since tryptophan concentrations are increased in plasma and brain in models of hepatic encephalopathy (Martin et al., 1983) and tryptophan loading causes hepatic coma in dog models of hepatic encephalopathy (Ogihara et al., 1966). There were similar findings in brain tissue from patients with hepatic failure, with a massive increase in CSF QA (sevenfold) and frontal cortex (threefold) as compared to controls. Two cortical levels were approximately 0.01 mM which is known to be neurotoxic in cultures (Whetsell, 1984). Like the animal models, tryptophan administration aggravated the hepatic coma and neurological symptoms (Sourkes, 1978). Further evidence suggested that administration of branched chain amino acids proved to be therapeutically useful in hepatic disease, as brain tryptophan levels were reduced (Rossi-Fanelli et al., 1982). Rats with hypoglycaemia have also demonstrated increased concentrations of QA in the brain and plasma (Heyes et al., 1990c). Increased concentrations of brain and blood QA have also been described in Lyme disease and meningitis and in a

chronically stimulated immune system (after administration of interferon) (Saito et al., 1991).

6.5 Our method for the determination of brain QA concentrations

The aim of our study was to investigate QA in the brain in HD, to provide evidence as to whether an increase in brain QA could be an aetiological factor in HD. QA concentrations were measured using a modification of previous GC-MS methods (Wolfensberger et al., 1983; Moroni et al., 1984a, 1986a). Concentrations were determined in the putamen and frontal cortex in tissue taken post-mortem from neuropathologically confirmed cases of HD and matched controls with no history of neuro-psychiatric disease, supplied by the Cambridge Brain Bank. Preparation and storage of tissue was as described previously (Spokes, 1979). All groups were matched for age, sex and post-mortem delay (Table 6.1). Statistical comparisons were made using Student's t-test on logarithmically-transformed data and Kendall's rank correlation.

6.5.1 Modification of previous GC-MS methods:

The method of Wolfensberger et al. (1983) was used initially, but this proved unsuccessful so each stage of the extraction and derivatization were tested.

Table 6.1

Details of subjects providing brain tissue post mortem: quinolinic acid

	Age (years)	Sex	Postmortem (hours)
Controls	65 ± 10	5M 4F	44 ± 31
Huntington's disease	64 ± 9	4M 5F	30 ± 26

6.5.2 Internal standard

An internal standard (IS) was used to increase the accuracy of the assay. Pyridine 3,4-dicarboxylic acid was tried, but this compound did not show the same derivatization properties as QA, whereas pyridine 2,4-dicarboxylic acid was found to act more like QA, and thus was employed. Concentrations of QA were calculated from the ratios of peak areas of QA and IS (respective retention times 5.6 and 4.8 min. using GC-MS).

6.5.3 Homogenization

Initially a variation on the Wolfensberger et al. (1983) method was used: 0.1 M perchloric acid (PCA) followed by rehomogenization in 0.1 M pH 9.4 disodiumhydrogen phosphate buffer (PB) resulting in approximately neutral pH. However QA was shown to remain in the pellet after centrifugation, even with rehomogenization (only 60% recovery of standards). Next 1% acetic acid was added to both the PCA and PB to displace QA in the pellet. This proved to be better (90-100% recovery), but elution produced a precipitate and wet residue which was impossible to dry down. This was suggested to be due to the PCA perhaps adhering to the column and then eluting in an insoluble form. Thus, other forms of homogenization were tested. Moroni et al. (1984a) used 0.3 M formic acid and this, followed by rehomogenization with 0.1 M PB, was found to be optimal. It was shown to produce no precipitate and dried down easily.

6.5.4 Extraction

The extraction of QA was tested initially using large (5-25 μg) amounts, and the efficiency was

monitored using either a spectrophotometer (wavelength = 275 nm; absorbance = 1) or a gas chromatograph with flame-ionization detection (GC-FID; 3% OV17 2 M column; GCQ support; 115°C; nitrogen = 13, hydrogen = 16, air = 10 psi). Pre-derivatisation purification of samples was necessary to remove any contaminants before injection into the GC-MS. Various processes were tested in order to selectively extract QA or IS, whilst allowing further purification stages to be applied. Ether and chloroform were found not to extract QA or IS at all. Solid phase extraction columns (Bond Elut 1 ml or 2.8 ml column volume) were also tried (NH2, C18, SAX); only the strong anion exchange (SAX) column was of use for the extraction of QA and IS. The application of standards to these columns resulted in negligible recovery, illustrating attachment to the column, whereas the other columns resulted in 100% recovery on the initial run through. Eventually a general washing procedure whilst QA and IS adhered to the SAX column, followed by specific elution, meant that further purification stages (e.g. passing through a C18 column first) were unnecessary.

Priming the column: methanol wash, followed by full blank extraction, followed by water wash, was found to produce ideal conditions for recovery of standards. This procedure was shown to remove interfering contaminants that produced abnormally increased recovery of standards (100-140%) from SAX columns.

6.5 5 Elution

Wolfensberger et al. (1983) used 0.1 M hydrochloric acid to elute the QA and IS from the column, which we tried initially (producing 94-100% recovery of

standards and samples, in a discrete volume). However, the wet residue and precipitate described earlier were impossible to dry down, therefore other protocols were tried in order to eliminate these problems. Methanol (50-100%) was used but produced negligible recoveries, as did acetic acid/ methanol combinations. Moroni et al. (1984a, 1986b) reported using 5 M formic acid, which proved more successful, especially in combination with methanol. Varying concentrations were tried, but 20% 1 M formic acid/80% methanol produced the best results for discrete elution in a small volume (1 ml). Recoveries varied from 100-140%, which prvided a further problem as there appeared to be increased concentrations of standards after passing through the column. This effect was negated by putting the column through a full blank extraction (no tissue, no standards) and 100% recoveries were achieved with such primed columns. It was found to be necessary to make the eluting solution fresh every day, as reduced recoveries (20%) were found to occur with old solutions. The columns were washed with water, 0.1 M formic acid, 25, 50, 80% methanol to remove any general contaminants prior to specific elution.

6.5.6 Transfer of eluate

The transfer of dried down eluate from plastic microtube to reactivial proved a difficult task and many different solvents were tested and appraised. Wolfensberger et al. (1983) reported using methanol, but in our hands there were was a loss of the standards or samples (recovery of 50%). Derivatization reagent solution was then tried with no success (30% recovery). Ammoniacal methanol or 20% formic acid/80% methanol were both tried producing more acceptable recoveries (84-100%). Ammoniacal methanol was used routinely as it was

found to be easier to dry down after transfer. However, there was a further problem with a white precipitate after transfer (associated with column elution). Eventually, to avoid any transfer losses, half of the eluate was dried directly in the reactivial, repeating the process with the remainder.

6.5.7 Derivatization

Wolfensberger et al. (1983) used 100 ul pentafluoropropionic anhydride (PFA) and 100 ul 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) to derivatize for 4 hours at 80°C. In our hands, this was found to produce inconsistent, incomplete derivatization. This was thought to be due to variables such as the time of reaction, temperature, tubes used, the type of reagent or the volume of reagent used. Thus each of these parameters was tested until optimal conditions were created for derivatisation. Moroni et al. (1984a) used HFIP in the presence of trifluoroacetic anhydride (TFAA) at 60°C for 1 hour, but this too resulted in low recoveries and inconsistencies. A time course tested derivatisation at 60°C and 110°C with maximal responses at 2 hours and 1.5 hours respectively. However, the higher temperature produced a greater, more consistent response, therefore this was used routinely. At this high temperature problems were encountered from leaky reactivials and distorted teflon seals and gas chromatography seals (due to the high internal pressure), which resulted in the solution drying out during derivatization. A combination of reactivials and three types of seals were eventually optimized. The effect of altering the volume of reagent was examined in order to reduce the pressure within the reactivial; the greater the volume used, the better response (with a

maximal effect with 100 μl).

6.5.8 Injection medium

Wolfensberger et al. (1983) used toluene to inject into the GC-MS, we tried heptane (50 μl) initially. There was a problem with 'dirty' samples and water (25 μl) was used to try to remove the 'dirt' into the aqueous layer. Latterly, heptane was used with 0.1 M ammonia present, as this combination was shown to reduce the solvent front and increase the response on the GC-MS. Conditions of the GC-MS were as follows: 1 μl solution was injected; 15 M x 0.32 mm chemically-bonded OV1 column at 120°C and a mass spectrometer to determine the ions at m/z 272, 300 and 448. The GC-MS analyses were carried out courtesy of Queen Charlotte's Hospital, London by John Halket.

6.5.9 Final method used:

Using 2,4-pyridine dicarboxylic acid as the IS, tissue (200 mg) was homogenized in 0.5 ml 0.3 M formic acid and centrifuged at 12000 g for 3 minutes. The pellet was resuspended in 0.5 ml PB (0.1 M, pH 9.4) and recentrifuged. The combined supernatants were centrifuged at 50000 g for 10 min and applied to a prewashed SAX column. After washing with consecutive 2 ml vols of water, 0.1 M formic acid and 25%, 50% and 80% methanol, QA and IS were eluted with 1 ml formic acid/methanol (20:80 v/v). This mixture was dried in a stream of nitrogen and derivatized by heating for 90 minutes at 110°C with 50 μl HFI and 50 μl TFA. This was dried under nitrogen and the derivatives dissolved in 50 μl heptane with 25 μl ammonia (0.1 M), prior to injection of 1 μl into the GC-MS (VG 7070 with Hewlett Packard mass selective detector).

6.5.10 Validation of method

Concentrations of QA were compared with results from a calibration curve, obtained by addition of QA (10-100 ng) to aliquots of a tissue homogenate and taken through the above procedure. Ordinary extracted and unextracted standard curves were also made. Multi-ion values were measured (m/z 272, 300 and 448) to assess the reproducibility of the method and blank extractions were run in each batch of samples. Standard curves were also produced for the spectrophotometer and GC-FID, whilst working up the method.

6.6 Results

6.6.1 Validation of extraction method

Linear standard curves were obtained for both the spectrophotometer and GC-FID for increasing concentrations of QA. Thus the efficiency of extraction and derivatization of large concentrations of QA was verified.

6.6.2 Validation of GC-MS method

Linear standard curves were produced for unextracted and extracted standards and for standards added to tissue. The extracted standards bore a closer relationship to the spiked tissue curve, than to the unextracted curve, therefore the QA concentration in tissue was calculated in reference to the spiked tissue calibration curve. Recovery of standards added to tissue in the calibration curve provided a measure of the precision of the method, with a coefficient of variation of 13%.

Table 6.2

Quinolinic acid concentrations in the brain in Huntington's disease

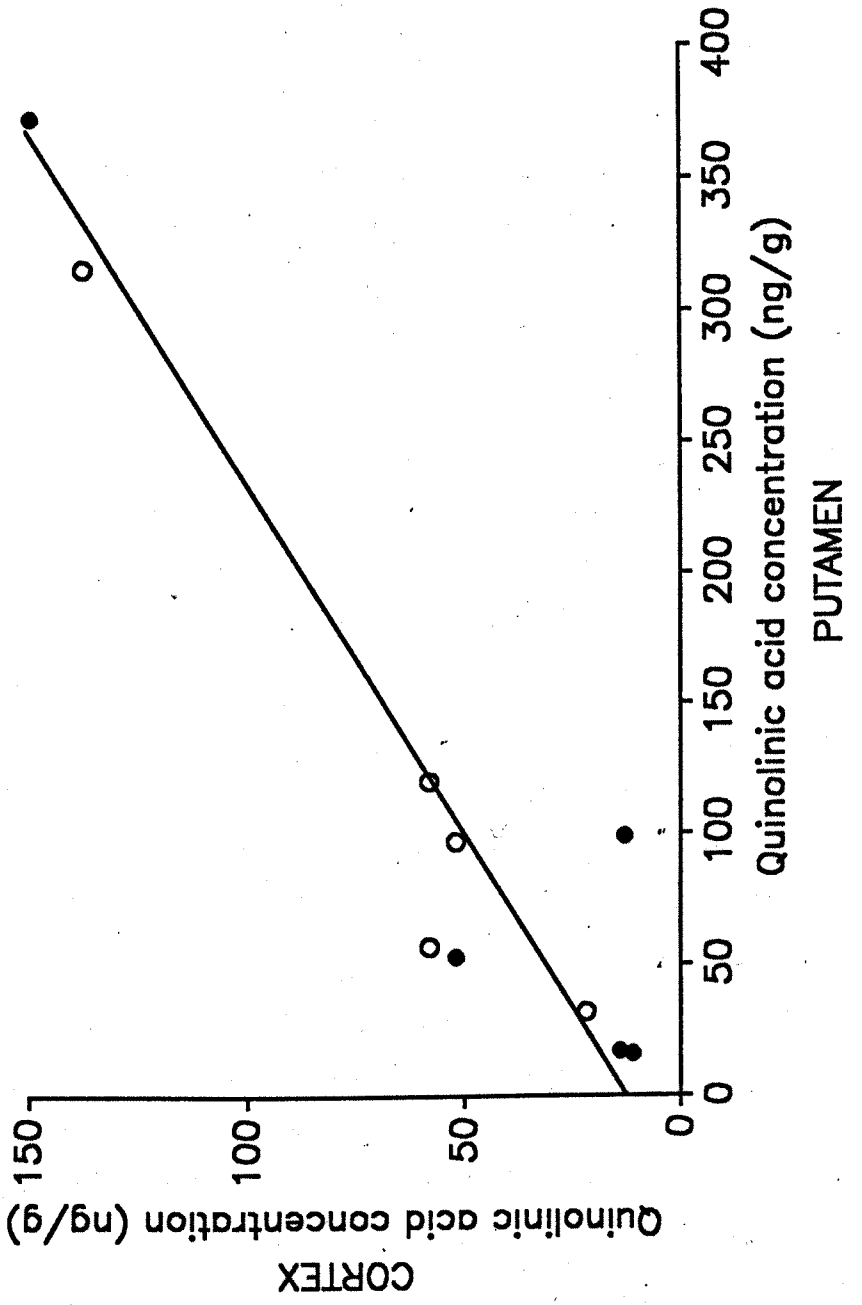
Values are means ± s.d. in ng/g tissue. Range in parentheses.

	Putamen	Frontal cortex
Controls	94 ± 37 (24 - 314)	48 ± 26 (22 - 149)
Huntington's disease	118 ± 31 (16 - 371)	65 ± 19 (11 - 149)

Figure 6.2

Correlation of quinolinic acid concentrations in the striatum and the cortex

● = controls
O = HD



6.6.3 QA concentrations in the brain in HD

Our results (Reynolds et al., 1988; Pearson and Reynolds, 1989) in Table 6.2 show that there is no significant difference in QA concentrations in HD or in controls, in either the putamen or the cortex. Furthermore there was no significant correlation with age, sex, post-mortem delay, nor within the HD group with duration of the disease. A significant positive correlation ($p < 0.01$) was shown between QA concentrations in the putamen and the cortex, indicating a consistency within each brain (Fig. 6.2). However there was a wide variation evident in brain QA between individuals. There was no significant correlation between QA and 5HT concentrations in the putamen.

6.7 Discussion

The results indicate that there is no significant increase in QA in HD reflected by post-mortem concentrations in the brain. Thus this provides no support for the hypothesis that increased QA is responsible for the neurodegeneration of HD. This finding is consistent wth CSF, urine and plasma measures of QA in HD (Schwarcz et al., 1988b; Heyes et al., 1985; Perry et al., 1987), none of which were increased above control values, although peripheral values, (see section 6.4) are unlikely to reflect brain values. Bruyn and Stoof (1990) observed that the 3-fold increase in QA concentration in AIDS CSF (Heyes et al., 1989b), provides further evidence that increased QA does not have an aetiological significance in HD. However the possibility of neurotoxic effects due to previous transient increases in QA cannot be excluded, especially in view of evidence (see section 6.3) that chronic exposure to only slightly

hyper-physiological levels of QA resulted in neurotoxic damage (Whetsell and Schwarcz, 1989). It is notable that there is a wide variation in brain QA between individuals, an observation also made by Wolfensberger et al. (1983). The factor responsible for this variation must be understood before any definite conclusions can be made, regarding the role of QA in the aetiology of HD. Despite the lack of change in brain QA in HD, there still remains evidence that the metabolism of tryptophan via kynurenine is abnormal in HD. The increased activity of 3HAO in HD brain (described earlier, Schwarcz et al., 1988a) is one example, although this has been postulated merely to reflect gliosis and not necessarily increased metabolism. Stronger evidence comes from a study by Connick et al. (1989) that measures KA in the brain in HD. Their hypothesis was that decreased concentrations of KA could have an aetiological effect in HD, due to its opposing effects to QA. Therefore normal concentrations of QA could have a relatively potentiated effect. However their results demonstrated increased KA in the motor cortex in HD. Thus further study of this system is indicated to establish whether there is an overactive metabolism, especially as some of the intermediates are also neuroactive (Lapin, 1981).

A recent report has just been published (Heyes et al., 1991b) that investigated the regional brain and CSF concentrations in HD. Their results are in concurrence with those from our study, as they found no significant changes in the putamen, cerebellum or frontal cortex (BA 10). However, there were slight reductions in most regions reaching significance in BA 17, 20, 28 as compared to control values. CSF QA values were also reduced (but not significantly) in HD and two early stage patients had similar QA

concentrations to the other HD cases. This was described as evidence that an early transient increase of QA was unlikely, but altered metabolism was still a possibility. The great variability of QA in the brain and CSF in HD and controls was again identified, but there were no significant effects of age, sex or post mortem delay. A further study by Beal et al. (1990a) reported QA concentrations as not significantly changed in the striatum in HD.

Chapter 7

Kynurenines

7.1 Tryptophan metabolism via the kynurenine pathway

7.1.1 Periphery

The metabolism of tryptophan, via the kynurenine pathway results in the formation of nicotinamide dinucleotide (NAD) and nicotinic acid, as well as other associated compounds from metabolism of intermediates. Activity and function of this kynurenine pathway has been documented in the mammalian liver (Fig. 6.1). Initially tryptophan is oxidised to N-formylkynurenine involving the enzyme, tryptophan pyrrolase (also known as tryptophan 2,3-dioxygenase). This process has been shown to be iron, copper and porphyrin dependent, as well as utilising molecular oxygen. The enzyme is inducible by tryptophan loading, corticosteroids and α-methyltryptophan (Sourkes et al., 1970), and can be inhibited by NADPH, via end product inhibition. N-formylkynurenine can be metabolised to either kynurenine (using the enzyme, kynurenine formylase) or anthranilic acid. Kynurenine hydroxylase catalyses the conversion of kynurenine to 3-hydroxykynurenine (3HK), using NADP and molecular oxygen (Battie and Verity, 1981). Alternatively, kynurenine can form anthranilic acid using the same enzyme (kynureninase) that catalyses the further metabolism of 3HK to 3-hydroxyanthranilic acid (3HA) (Inada et al., 1984; Takeuchi et al., 1980; Kawai et al., 1988). Kynureninase is vitamin B6 dependent, as is kynurenine transaminase, the enzyme involved in the conversion of kynurenine to kynurenic acid (KA), as well as the production of xanthurenic acid from 3HK. There is further metabolism of 3HA, via

quinolinic acid (QA), to NAD and nicotinic acid (see section 6.1) (Stone and Connick, 1985; Gal and Sherman, 1975).

7.1.2 Brain

There has been speculation as to whether the same pathway exists (or indeed functions) in cerebral tissue. Evidence suggests that most of the necessary enzymes are present in the rat brain (Okuno et al., 1990; Kohler et al., 1988b; Schwarcz et al., 1988a; Battie and Verity, 1981; Kawai et al., 1988) and several intermediate compounds have been located (Moroni et al., 1988a, 1988b; Joseph et al., 1979; Guilarte and Wagner, 1987). Gal and Sherman (1978) made a study of kynurenine synthesis and metabolism in rat brain. They made intraperitoneal (i.p.) injections of radiolabelled tryptophan and demonstrated, for the first time, the presence in the brain of anthranilic acid, 3HA, xanthurenic acid, KA and quinaldic acid. Earlier work (Gal, 1974) suggested the presence in rat brain of a similar (but not identical) enzyme to tryptophan pyrrolase: indoleamine 2,3-dioxygenase. This brain enzyme appeared to respond to different factors, thus it was affected by tryptophan loading, but not cortisol. Also, the brain enzyme is reported to be nonspecific for tryptophan, having other substrates including 5-hydroxytryptamine (5HT), melatonin and 5-hydroxytryptophan (5HTP) (Stone and Connick, 1985). The question still remains as to whether the kynurenines present in the brain are being formed locally, or being transported from the periphery through the blood-brain barrier. Furthermore, anthranilic acid has been shown to be the preferred precursor for the production of 3HA, compared with 3HK, in the rat brain (Baran and Schwarcz, 1990). Also, anthranilate hydroxylase

(the enzyme catalysing the production of 3HA) has been identified in rat brain (Baran and Schwarcz, 1990).

Initial evidence suggests that kynurenine and 3HK are able to cross this barrier (Stone and Connick, 1985), and furthermore, KA has been demonstrated to be synthesised in the brain (Swartz et al., 1990b), from kynurenine transported across from the periphery. They used microdialysis techniques to assess whether KA was synthesised in situ in the brain, or whether it was transported from the periphery. Systemic administration of kynurenine (or tryptophan) was demonstrated to produce increased concentrations of KA in the rat brain. However, only kynurenine produced this effect when applied directly into the brain. They suggested that kynurenine (and not tryptophan) was the major precursor of KA, and was transported through the blood-brain barrier from the periphery. The effect could be blocked by using a kynurenine aminotransferase inhibitor. They also suggested that if the QA in the brain is not derived from local metabolism of kynurenine (but instead originates via the anthranilic acid/3HA pathway, as described above), then it may be possible to reduce the peripheral kynurenine/QA pathway selectively by giving kynureninase inhibitors. Thus systemic QA effects could be reduced, and more kynurenine would be available for transport into the brain (thus increasing cerebral KA concentrations, if required). These general effects were consistent with findings (Speciale et al., 1990) that also demonstrated that administration of aminooxyacetate (the unspecific transaminase inhibitor) resulted in marked reductions of extracellular KA in rat brain. In addition, administration of kynurenine to the striatum resulted in increased concentrations of KA.

Furthermore, they suggested that intrastriatal injections of aminooxyacetate in rat can produce neurotoxic degeneration and seizures (similar to QA). They hypothesised that this was evidence for an indirect link between modulation of KA and excitotoxic damage.

Speciale and Schwarcz (1990; et al., 1989a) studied the uptake of kynurenine into rat brain slices (and into astrocytes). They described two components of uptake: sodium-independent and sodium-dependent. The former was suggested to be using the high-affinity saturable uptake transporter of neutral amino acids and was primarily localised to astrocytes. The latter non-saturable process was localised more in neurones, and regulated by extracellular sodium levels. Furthermore, 3HK was found to compete with kynurenine for uptake into cells (both with and without the presence of sodium).

More evidence comes from work by Fukui et al. (1991). They made an extensive study to determine brain uptake and blood-brain transport of kynurenines, to try to separate extra-cerebral synthesis from local metabolism in the rat brain. Their results indicated that kynurenine (and to a lesser extent 3HK) is taken up into the brain via the large neutral amino acid carrier system (L-system), and anthranilic acid via significant passive diffusion. However, peripheral 3HA, KA and QA were found not to cross the blood-brain barrier easily, therefore were suggested not to contribute to brain pools. Anthranilic acid (unlike kynurenine) was suggested to be affected by the amount of plasma protein binding. As described earlier, anthranilic acid has been shown to be the preferred precursor for 3HA in the rat brain.

Kynurenine has also been demonstrated to produce anthranilic acid in rat brain homogenates (Kawai et al., 1988) and they suggested that the activity of kynureninase (1.5% of liver activity) is reportedly low in rat brain (as is the activity of kynurenine hydroxylase). The optimal substrate for kynureninase is 3HK as compared with kynurenine (15:1 activity ratio) (Kawai et al., 1988; Inada et al., 1984; Inada et al., 1984; Battie and Verity, 1981). Okuno et al. (1991) described the presence of two kynurenine aminotransferases in the human brain, with different characteristics to each other, and to the similar enzyme identified in rat brain astrocytes (Okuno et al., 1990). These two enzymes both catalysed the production of KA, but had different sensitivities to amino acids, as well as different kinetic characteristics.

Moroni et al. (1988a) demonstrated a circadian rhythm for KA in rat brain. Concentrations were three times greater at midday than at midnight. They also showed that brain KA levels were increased with age. A further study (Baran and Schwarcz, 1990) examined the effects of administering an inhibitor of 3HAO (4-Cl-3HA); they found rat brain concentrations of 3HA were increased. Heyes et al. (1989a) demonstrated that systemic tryptophan loading resulted in increased concentrations of 3HK, QA, 5HT and 5-hydroxyindoleacetic acid (5HIAA). A further report from Beal et al. (1991a) described results indicating that excitotoxic lesions produce increased brain concentrations of KA and increased responses to loading with precursor, on the formation of KA (Turski et al., 1989).

7.2 Neuroactive characteristics of kynurenines

Many of the kynurenine metabolites exhibit neuroactive properties. Lapin (1981) made an extensive review of the subject, investigating the production of seizures following administration of some kynurenines. Intracerebroventricular injection of QA, KA , 3HK, and kynurenine produced seizures in mice (in order of potency, with QA having the lowest threshold value). 3HA, xanthurenic acid, nicotinic acid and picolinic acid were less effective, with anthranilic acid totally ineffective. Peripheral injections were generally not effective (except for QA), although immature animals were susceptible to i.p. administration. This was suggested to illustrate the difference in the permeability of the blood-brain barrier, which was also immature and therefore not a selective barrier. There were also qualitative differences in the response to individual kynurenines. Thus, kynurenine itself had a short latency and produced a characteristic unique myoclonus and locomotor hyperactivity unlike that produced by any of the other kynurenines. These myoclonic seizures were blocked using serotonergic drugs, which are also known to prevent myoclonus clinically (Magnussen et al., 1977). Also, tryptophan has been used to prevent epileptic seizures, but the evidence has been contradictory as it appears both to inhibit and initiate fits. Lapin (1981) described this to be an example of the balance between the production of 5HT and the kynurenines. The effects of antagonists were considered; thus, the convulsant effects of kynurenine were selectively antagonised by taurine, less selectively by glycine, with GABA and muscimol having no effects (although the actions of traditional anti-convulsants were varied). The actions of administering 3HK, 3HA and KA were not

described quantitatively, although the effects of QA injections were compared with those of kynurenine (see section 6.3). Vecsei and Beal (1990) reported intracerebroventricular injections of kynurenine or KA in rats. They described ataxia and stereotypic behaviour with muscular hypotonia only after the KA injection, and slight behavioural changes after kynurenine injection. A further study of behavioural actions of KA was described by Schmitt et al. (1990). They injected KA into the dorsal periaqueductal gray matter of rats, where it was observed to have anxiolytic actions. AP7 was described as having similar actions. Lapin and Rysov (1990) investigated the effects of catecholaminergic drugs on QA and kynurenine-induced seizures. Kynurenine-induced seizures were demonstrated to be increased with anti-dopaminergic drugs (reserpine, haloperidol) and prevented with dopamine itself. However, QA-induced seizures were affected to a lesser degree by the anti-dopaminergic drugs, and not at all by dopamine. Also, apomorphine, amphetamine, noradrenaline, but not serotonin were all anticonvulsant for kynurenine, but not QA. Therefore each compound has its own particular profile and characteristic mechanisms. A further study by Lapin and Rysov (1989) suggested that kynurenine-induced seizures involve the GABA receptor, the strychnine-insensitive glycine site, and the picrotoxin-barbiturate subunit of GABA-benzodiazepine-chloride channel complex. Guilarte et al. (1987) reported that 3HK (1 mM) significantly decreased the benzodiazepine receptor binding affinity ((^{3}H) flunitrazepam) in rat brain and lower (Ki = 0.2 μM) concentrations of 3HK inhibited the effects of GABA stimulation on this binding.

7.2.1 Neurotoxicity of kynurenines

The neurotoxicity of the kynurenines has been little studied, although there is one study made by Eastman and Guilarte (1989) on the effects on neuronally-derived cells in culture. Cytotoxicity was quantitated by determining the release of lactate dehydrogenase. Exposure to kynurenine, QA, KA, 3HK and xanthurenic acid was compared to that produced by a predictable neurotoxic agent, glutamate. Significant toxicity was only seen with 3HK and xanthurenic acid (with 100 μM 3HK the threshold dose). 3HK neurotoxicity was 1-2 orders of magnitude greater than glutamate and the dissimilar logarithmic dose response curves were suggested to be good evidence that glutamate and 3HK had different mechanisms of toxicity. Indeed the well established neurotoxic effects of QA were absent and thought to be due to the lack of NMDA receptors on these neurones. The 3HK toxicity was shown to have delayed effects: 8-12 hours exposure produced the peak response for chronic experiments, but a shorter exposure (2 hours) whilst having no acute effects, caused cell lysis 22 hours later. Morphologically, the cells retracted their processes, became more shrunken, rounded and detached from the culture dish. The lack of response to kynurenine was explained to be due to the absence of a ring hydroxyl group, which would form a quinoid species (e.g. quinoneimine). The neuroprotective effects of antioxidants and taurine were examined (superoxide dismutase (SOD), catalase, glutathione, mannitol). Glutathione, catalase or (SOD + catalase) together, were found to be protective, but mannitol (a hydroxyl radical scavenger) and taurine were ineffective. Thus, they suggested that hydrogen peroxide could change 3HK to a quinoneimine by oxidation (Tomada et al., 1986). In a further

study, Eastman and Guilarte (1990) suggested that hydrogen peroxide plays an important role in the neurotoxicity of 3HK. 3HK toxicity is delayed after initial exposure, which they suggested reflected an 'ongoing catalase-sensitive toxic process'. Catalase attenuates the process, therefore it cannot be irreversible. Two possible modes of action were postulated: direct toxicity of hydrogen peroxide, or hydrogen peroxide may oxidise 3HK to a toxic metabolite. Increased intracellular peroxidase activity attenuates 3HK toxicity, thus the former mode of action was implicated. Further evidence was that horseradish peroxidase pretreatment (that increases 3HK metabolism), reduced toxicity. Catalase was demonstrated to reduce 3HK toxicity post treatment (after removal of extracellular 3HK and hydrogen peroxide), suggesting that detoxification of intracellular hydrogen peroxide is associated with reduced toxicity. Also, desferrioxamine, an iron chelator, was shown to reduce the toxic effects of 3HK.

There is extensive literature describing the neuroactive actions of KA, particularly in regard to its opposing effects to neurotoxins (including QA) (see section 6.3). It has been described as a 'broad spectrum antagonist of excitatory amnino acid receptors, antagonising the electrophysiological responses to NMDA, kainate and to a lesser extent quisqualate receptor agonists'(Swartz et al., 1990a). At the NMDA receptor complex, KA is thought to act both at the agonist site and at the glycine allosteric site, where it is several times more potent (Danycz et al., 1989). It has been suggested that altered endogenous concentrations of KA may modulate the effects of neurotoxins in such neurological disorders as Huntington's disease (HD), stroke, hypoglycaemia or hypoxia. Indeed, co-

injection of KA was shown to block the neurotoxic effects of striatal QA in an animal model of HD, and blocked the seizures induced by the administration of QA into the rat hippocampus (Foster et al., 1984b). Further effects were to reduce the neuronal degeneration seen in an animal model of ischaemia (pre-treatment only) (Germano et al., 1987) or to protect against biochemical or behavioural changes as a consequence of QA administration into the nucleus basalis (Boegman et al., 1985; Wirsching et al., 1989). Jhamandas et al. (1990) compared the in vivo and in vitro modulation by kynurenines of QA-induced damage of the nucleus basalis. 3HA actually produced neurotoxic changes similar to QA, but with less potency. The mechanism for this was described as unknown, but may be due to 3HA being converted to QA in the homogenate, or alternatively 3HA might be auto-oxidised to form a free radical species. They thought it unlikely that 3HA was acting via the NMDA receptor directly. Co-injection of other metabolites (together with QA) resulted in protection (KA > picolinic acid > quinaldic acid > anthranilic acid).

7.3 Previous methods of determining concentrations of kynurenines

Previous methods have enabled the measurement of 3HK in urine (Yeh and Brown, 1977), rat plasma (Allenmark et al., 1984) and animal brain tissue (Heyes, 1988), initially using ion-exchange with colorimetric detection and latterly high-performance liquid chromatography (HPLC) with electrochemical detection (ED). Analysis is often preceded by an extraction process to remove any contaminants (e.g. uric acid).

7.4 Our method for the determination of brain concentrations of 3HK

We devised a simple HPLC-ED method used for the determination of 3HK in human post-mortem brain tissue, and involving a straightforward extraction of human plasma (which is also applicable to brain if necessary).

7.4.1 Direct method (Pearson and Reynolds, 1991)

Brain tissue (50-100 mg) was homogenised in 0.5 ml of 0.1 M perchloric acid (PCA) containing 100 μM ascorbic acid and centrifuged at 12000 g. Refrigerated stock solutions of 3HK (1 mg/ml in 0.1 M hydrochloric acid with 100 μM ascorbic acid) were diluted daily to 20 ng/ml with PCA. Aliquots of standards or sample supernatant were injected directly into the HPLC system.

7.4.2 Extraction method

This was used for measurment of 3HK in plasma (and brain tissue if 3HA determination was required). Equal volumes of plasma and PCA were centrifuged at 12000 g for 3 minutes to produce a supernatant for extraction. Alternatively, supernatant from brain tissue (prepared as described above for the direct method) was extracted in the same way as plasma supernatant. Thus, 200 μl supernatant were mixed with 50 μl of PCA and 250 μl of 0.1 M phosphate buffer (PB) (pH 4.0), resulting in a pH 2.3 solution. This was then added to a strong cation-exchange (1cc Bond Elut SCX) column which had been pre-washed with 1 ml of methanol, 1 ml of 50% methanol-pH 2.3 solution, 4 ml of water and 1 ml of pH 2.3 solution. Aliquots (0.5 ml) of 50% methanol-pH 2.3 solution and water washed the column prior to

elution with 1 ml of 0.1 M PB (pH 7). All the solutions contained 100 μM ascorbic acid. Standard solutions (0.5 ml of 10 ng/ml at pH 2.3) were extracted in the same way as the samples.

7.4.3 HPLC system

Aliquots (20 μl) of the unextracted standard solutions and prepared extracts of standards and samples were injected onto the column. Isocratic reversed-phase separation was achieved using an ODS-2 5 uM column (25 cm x 4.6 mm I.D.) at 40°C, with a 0.1 M phosphate/acetate buffer at pH 3.2 containing 2.5 mM octylsulphonate and 0.05 mM EDTA (flowing at 1.0 ml/minute). Quantification used an electrochemical detector (BAS) containing a glassy carbon electrode set at 0.60 V (versus Ag/AgCl). Statistical comparisons were made using the Student's t-test of logarithmically-transformed data.

7.4 4 Validation of the method

Fig. 7.1 illustrates typical chromatograms from a directly injected standard and an extracted brain samples. In the samples, a single peak was demonstrated for 3HK (retention time = 10.4 minutes) which was verified by comparison with the retention time of authentic standard peaks, under varying chromatographic conditions. Recovery from standards added to samples was 97 ± 2% (coefficient of variation, n = 5) for unextracted samples and 100 ± 4% (n = 5) for extracted samples. Also, oxidation curves were performed by measuring the peak height at varying voltages (0.4-0.9 V). These proved similar for the standard, sample and sample with added standard, with 0.6 V providing the optimal conditions.

Figure 7.1

HPLC chromatogram of 3-hydroxykynurenine and 3-hydroxyanthranilic acid

A shows a typical brain sample; B shows a typical standard (10ng/ml). Full scale deflection = 1 nA

1 = 3HK
2 = 3HA

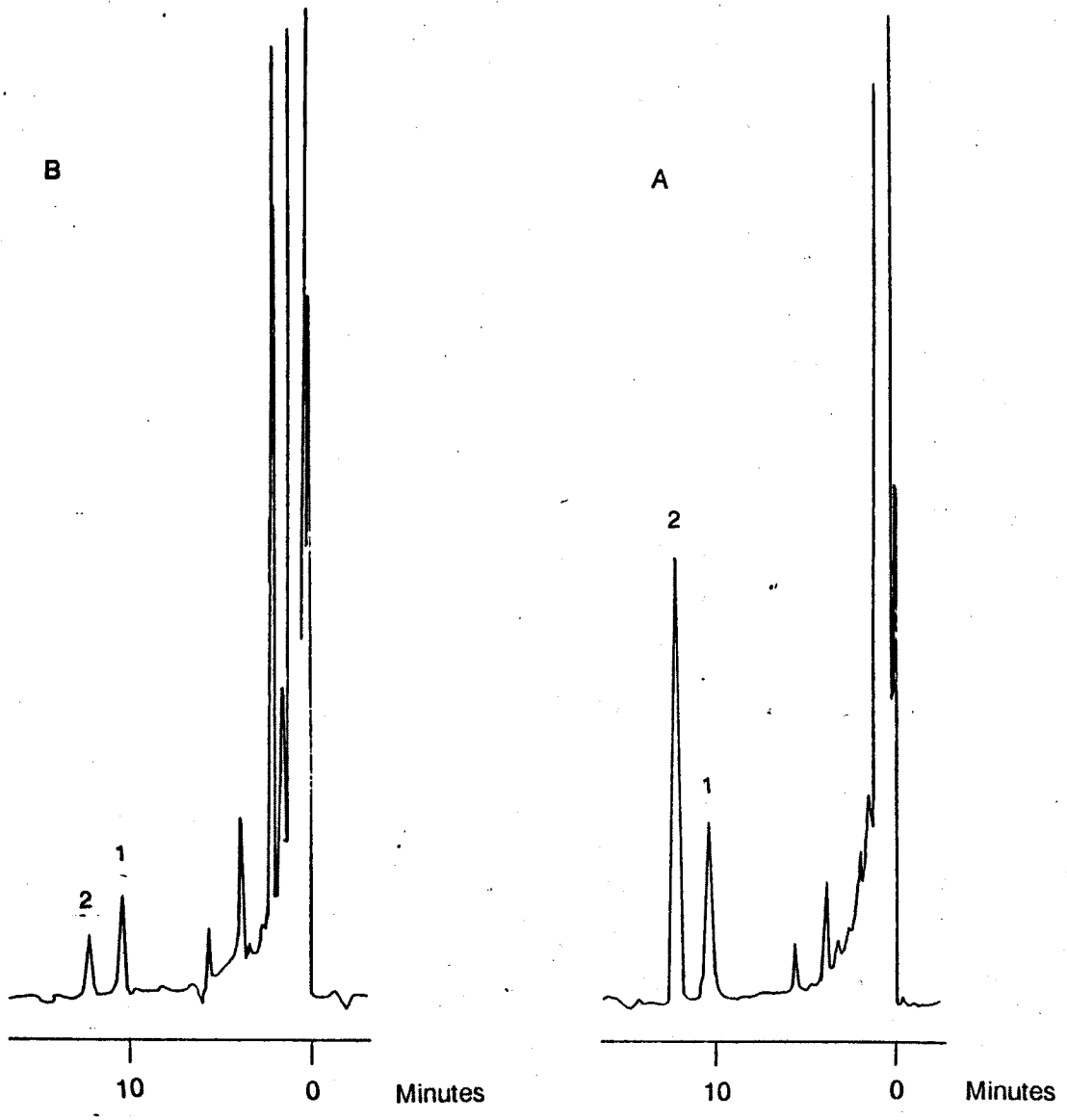
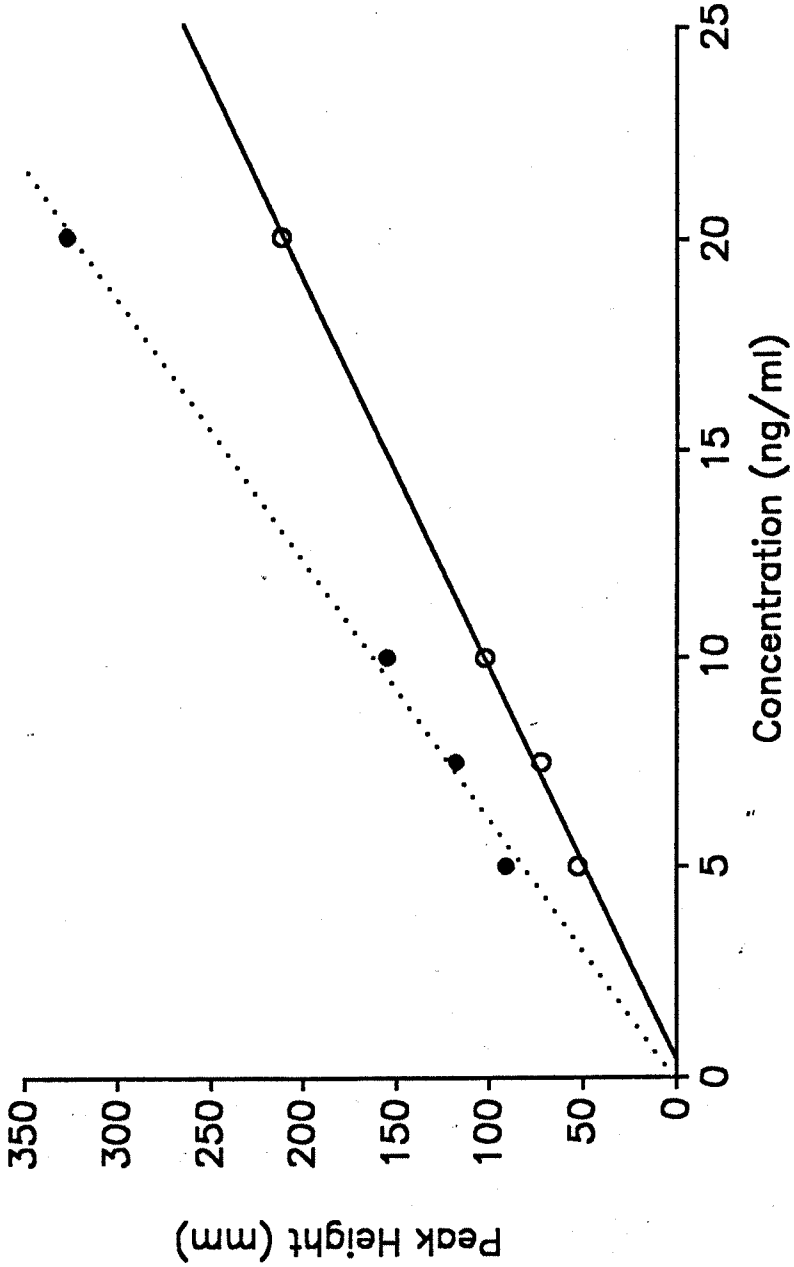


Figure 7.2

Standard curve of 3-hydroxykynurenine and 3-hydroxyanthanilic acid concentrations

Hatched circles = 3HA
Open circles = 3HK



Linear standard curves for concentration (0-20 ng/ml) versus response were demonstrated for both unextracted and extracted 3HK (Fig. 7.2). The limit of detection was approximately 40 fmol 3HK on the column. Replicate injections (n = 4) of 20 μl gave a 1.0% coefficient of variation. 3HK samples and standards were shown to be stable over 18 hours if kept at 4°C, but only when ascorbic acid was present. The presence of ascorbic acid at all stages was found to be a prerequisite for good recovery of extracted 3HK, preventing degradation on the SCX column.

A related kynurenine, 3HA, could also be determined using this system. However, measurement of 3HA without extraction was impossible due to coeluting peaks, and although the extraction process was applicable to 3HA in the brain, plasma 3HA still contained interfering peaks even after extraction. Similar validation to the above confirmed the identity of the peak in the brain with a retention time of 12.2 minutes (see Fig. 7.1).

Concentrations of 3HK and 3HA were calculated from comparisons of sample peak height, corrected for dilution.

7.5 Our study

In recent years the kynurenine pathway of tryptophan metabolism has attracted much interest, especially as some of these compounds are neuroactive (see section 7.2). Neurotoxin-induced neuronal degeneration has been implicated in the aetiology of a wide range of neurological conditions (temporal lobe epilepsy, glutaric aciduria, HD, febrile convulsions, AIDS, ischaemia and hypoxia) (Heyes et al., 1989a; Stone and Connick, 1985). In this

respect, the involvement of glutamate receptors, particularly the NMDA receptor complex, is well documented. QA and KA have been shown to have opposing actions mediated through this complex (Swartz et al., 1990a). Over the last decade the QA hypothesis of HD has produced the animal model with the most similarities to HD (see section 6.3), although there are some discrepancies. It was suggested that increased concentrations of the endogenous neurotoxin, QA, may be involved in the pathogenesis of HD. However, we have shown (see section 6.7) this not to be true, as brain QA in HD was unchanged from control values. Another kynurenine metabolite, 3HK, has demonstrated neurotoxic properties when applied to neuronal cell cultures (Eastman and Guilarte, 1989) and intracerebroventricular injections of 3HK have been shown to induce seizures (Lapin, 1981). Also, in vitamin B6 deficient neonatal rats, increased brain concentrations of 3HK have been reported (Guilarte and Wagner, 1987). As one consequence of vitamin B6 deficiency is loss of striatal GABAergic neurones (Wasynczuk et al., 1983), we hypothesized that 3HK may be of interest in HD where there are similar cell losses. Furthermore, Heyes et al. (1989a) postulated a role for 3HK in the aetiology of febrile convulsions, glutaric aciduria and the neuropathology of AIDS. Our study was designed to find out whether concentrations of 3HK (or any other associated tryptophan metabolites) were changed in the brain from patients with HD. Furthermore we aimed to identify any factors associated with changes in 3HK. Thus, we made a similar study in Alzheimer's disease (AD) brain tissue (using matched controls), where neuronal degeneration also occurs, to assess the effects of tissue atrophy. Concentrations of 3HK were determined in the frontal and temporal cortex and putamen from the HD group

and matched controls, and in the temporal cortex from the AD group and controls to match. In order to understand more about the metabolic pathway, we also determined concentrations of 3HA and tryptophan in the brain in HD. Tryptophan concentrations were measured using an adaptation of the HPLC-ED method (see section 2.3).

Finally, we made a preliminary study to investigate the possibility of a peripheral marker for HD, by determining the plasma concentrations of 3HK and tryptophan from patients with HD and matched controls.

Brain samples (supplied by the Cambridge and Nottingham Brain Tissue Banks) were obtained post-mortem from patients with neuropathologically-confirmed HD or AD, and from matched controls with no previous history of neuropsychiatric disease. Preparation and storage of brain tissue was as described previously (Spokes, 1979).

7.6 Results

The results from our study in HD (Reynolds and Pearson, 1989) (Table 7.1) show 3HK concentrations to be significantly increased in the putamen (342% of mean control value) and two cortical regions (169% and 282% of mean control values). 3HA concentrations in the frontal cortex in HD were not significantly changed from control values. Tryptophan concentrations were slightly increased in the HD brain, significantly in the temporal cortex, as compared with controls.

Table 7.1

Tryptophan and metabolites in the brain in Huntington's disease

Values are means ± s.d. in ng/g tissue (3HK, 3HA) or μg/g tissue (tryptophan). *p < 0.05, **p < 0.01, ***p < 0.001

	Temporal cortex	Frontal cortex	Putamen
3HK			
Controls	65 ± 56 (n=20)	33 ± 26 (n=21)	19 ± 14 (n=21)
Huntington's disease	110 ± 47** (n=18)	93 ± 60** (n=22)	65 ± 47*** (n=19)
3HA			
Controls	-	12 ± 7 (n=13)	-
Huntington's disease	-	19 ± 15 (n=11)	-
Tryptophan			
Controls	10 ± 4 (n=16)	-	18 ± 7 (n=14)
Huntington's disease	16 ± 7* (n=12)	-	27 ± 12 (n=11)

Table 7.2

3-Hydroxykynurenine concentrations in the brain in Alzheimer's disease

Values are means ± s.d. in ng/g tissue.

	Temporal cortex
3HK	
Controls	65 ± 33 (n=12)
Alzheimer's disease	82 ± 41 (n=12)

Table 7.3

Tryptophan and 3-hydroxykynurenine concentrations in plasma in Huntington's disease

Values are means ± s.d. in ng/ml (3HK) or μg/ml (tryptophan)

3HK	
Controls	7.3 ± 4.1 (n=7)
Huntington's disease	6.7 ± 2.2 (n=7)
Tryptophan	
Controls	10.6 ± 1.2 (n=7)
Huntington's disease	10.2 ± 2.4 (n=7)

A significant ($p < 0.001$) positive correlation was evident for cortical and striatal 3HK values in the brain in HD and controls. However, no relationships were demonstrated between tryptophan and 3HK levels and there was only a weak correlation between 3HK and 3HA. There were no significant effects of age, sex, post-mortem delay or agonal state, or within the HD group, age at onset or duration of disease. Furthermore, it appears that any group differences are not associated with prior medication (e.g. tetrabenazine or neuroleptics). In AD cortical 3HK was slightly increased (Table 7.2), although this was not significantly changed from matched controls or HD values. The preliminary study determining plasma concentrations of 3HK and tryptophan, showed no significant changes from control values (Table 7.3).

7.7 Previous studies in the brain in HD

Previous investigations have been made to determine the concentration of kynurenines in the brain in HD. Connick et al. (1988; 1989) using a method verified by Moroni et al. (1988b), measured KA concentrations in the caudate, pallidum, frontal cortex and motor cortex from patients dying with HD and controls. They reported a general increase throughout the brain in HD, only reaching significance in the motor cortex (BA 4). However, only 4 control cases were used in the BA 4 comparisons, thus statistical analysis is not really possible. The reported increases contradicted their original hypothesis that predicted decreased levels of KA causing a shift of the QA/KA balance, resulting in neurotoxicity. They speculated that increased KA in the pallidum may be associated with the production of chorea, but correctly mentioned that the levels used experimentally (Robertson et al., 1988) were

much higher than the levels they found. Contrasting evidence comes from another study by Beal et al. (1990a). They measured concentrations of 25 compounds, mainly components of tryptophan and tyrosine metabolism in the putamen in HD. Tryptophan, kynurenine, KA, 3HA and 3HK concentrations were shown not to be significantly changed, whereas 5HT, 5HIAA and 5HTP concentrations were significantly increased (by 50%). They also reported a significantly increased ratio of kynurenine to its metabolites in HD, but a decreased tryptophan to its metabolites ratio. They described this as evidence for increased tryptophan metabolism, but decreased kynurenine metabolism in HD. Correlations were evident between tryptophan and metabolites (particularly kynurenine) and these correlations were generally greater in the brain in HD. They suggested that this was evidence for increased substrate-driven pathways in HD. Also, they reported discrepancies between the absolute values of control KA in their study, compared with Turski et al. (1988) and Connick (1988; 1989), which may explain the contrasting results described in the HD brain. CSF concentrations of KA were also measured by Beal et al. (1990a) in HD, but these were not significantly changed from control values. Tryptophan concentrations have been determined in many investigations of amino acids in HD. Perry et al. (1973) reported no significant changes, as did Beal et al. (1990a). Yates et al. (1973) described plasma tryptophan levels as not significantly changed in HD, however, there was a significant reduction in plasma from fasted patients. Also, Phillipson and Bird (1977) found total plasma tryptophan not to be significantly changed in HD, but free plasma tryptophan was reduced (in fasting and hypoglycaemic states). This was in association with increased plasma non-esterified fatty acids

(which compete with tryptophan for albumin plasma sites, that were reportedly not significantly changed). These findings, as well as lowered concentrations of plasma neutral amino acids in HD (that compete with free tryptophan for transport into the brain) were suggested to be consistent with increased tryptophan transport into the brain in HD. The abnormal growth hormone found in plasma from fasting HD patients, further compounds the effects (Phillipson and Bird, 1976) (as it affects the release of non-esterified fatty acids from peripheral deposits). They suggested that in HD there is a similar pattern to that seen in 'metabolic starvation'.

7.7.1 Other clinical studies of kynurenines

Tryptophan metabolites (including 5HT and kynurenine) were determined throughout the brain in schizophrenia, with no significant changes from control levels reported by Joseph et al. (1979). Administration of systemic endotoxin was reported to increase tryptophan, 5HIAA, 3HK and QA in the mouse cortex (Heyes et al., 1989a), as well as increasing urinary kynurenine and xanthurenic acid (Rapoport and Beisel, 1971). Also, γ -interferon (chronically adminstered) results in increased brain QA, as well as increases of kynurenine and QA concentrations in plasma (Saito et al., 1991). Increased concentrations of KA have also been reported in the CSF of HIV-1 patients, septicaemia and retrovirus infection (Heyes et al., 1991a; Heyes and Lackner, 1990; Heyes et al., 1990b) and CSF concentrations of kynurenine and QA were also increased in septicaemia. Kynurenine was not significantly altered in the superficial layers of the spinal dorsal horn in normal and arthritic rats (Godefroy et al., 1990). The effects of drugs on kynurenine

metabolism were studied in vivo and in vitro (Mostafa et al., 1982); phenothiazines and sulpiride were administered to rats. Phenothiazines were shown to increase activity of kynurenine hydroxylase, but decreased activity of kynurenine transaminase. Sulpiride was without a significant effect on either enzyme. However, in vitro, only the increased effects with kynurenine hydroxylase was seen. Kynurenine transaminase was not significantly affected, and again sulpiride had no significant effects on either enzyme. 3HA has been suspected to have a possible aetiological role in bladder cancer which may be associated with mitochondrial dysfunction (Teulings et al., 1973; Quagliarcello et al., 1964). Manthey et al. (1990) suggests that auto-oxidation of 3HA with molecular oxygen may form free radical species involved in the induction of bladder cancer. 3HK levels have also been found to be increased in urine from patients with bladder cancer (Abdul-Fadl and Khalafallah, 1961). Kynurenine has been reported to be increased in diseases such as hepatic encephalopathy and diabetic coma (Kornhuber et al., 1988). Occasional cases of hydroxykynurenuria have been described which demonstrate increased urinary excretion of kynurenine, 3HK and xanthurenic acid. Symptoms included chronic stomatitis, ulcerated gums, gingivitis and mental retardation that did not respond to vitamin B complex administration (or vitamin B6 alone). An absence of kynureninase was hypothesized in these cases (Reddi, 1978). Plasma tryptophan concentrations have been reported to be decreased in depression (Hoes and Loeffen, 1981), as has a dysfunction of urinary excretion of xanthurenic acid (Hoes and Sijben, 1981). Also kynurenine has been described as being reduced in CSF in epilepsy (Young et al., 1983). Freese et al. (1991) reviewed the impact of kynurenines in

neurological disorders discussing HD, glutaric aciduria, hepatic encephalopathy and porphyria (as porphyrin is a co-factor for tryptophan pyrrolase).

7.8 Discussion

Our results (described earlier) provide evidence for a dysfunction in tryptophan metabolism in HD. For the first time we have shown elevated 3HK concentrations in all the regions measured, suggesting a general increase throughout the brain in HD. This is consistent with the results of Connick et al. (1989) that produced evidence for increased tryptophan metabolism (KA) in the motor cortex in HD. However, the study by Beal et al. (1990a) suggested that the opposite may occur. They hypothesized reduced kynurenine turnover in HD but found no significant changes in concentrations of striatal tryptophan or kynurenines (including 3HK, 3HA or KA). Thus, our results contradict their findings and furthermore, the control values for striatal KA reported previously in studies by Turski et al. (1988) and Connick et al. (1989a) are quantitatively different. Measuring 25 compounds from one sample may increase systematic errors and an overall ratio accuracy of ± 20% was described. In the same study HVA concentrations were not significantly altered in the brain in HD, which is also contrary to our, and other, findings.

The tryptophan values are consistent with previous studies (Perry et al., 1973; Bonilla et al., 1988), except for the new finding of an increase in the temporal cortex in HD. Brain 3HA concentrations have not been reported before in controls or in HD, except for the Beal et al. (1990a) study of the striatum.

The lack of a significant increase of cortical 3HK in AD suggests that the effect in HD may not be solely related to tissue atrophy, and may reflect a genuine dysfunction of tryptophan-kynurenine metabolism. Furthermore, the magnitude of the increase of 3HK in HD is similar in both the cortex and the striatum, which show varying degrees of tissue shrinkage. The absence of any effects with factors including age, post mortem delay, agonal state or drug status is important, as the groups are inevitably not controlled. In particular, more cases in the HD group died from pneumonia, as compared with the control group, and the HD patients were mainly taking neuroleptics or tetrabenazine before death. However, Beal et al. (1990a) also reported that concentrations of kynurenines were not significantly affected by age, post mortem delay, agonal state or drug status.

The strong correlation between cortical and striatal 3HK concentrations (in both controls and in HD) provides evidence to validate the methods used. The control of brain tryptophan metabolism is not well understood, and the lack of any direct correlation between 3HK and tryptophan concentrations suggest that this is not a simple relationship. Systemic tryptophan loading is known to result in increased brain concentrations of 3HK (as well as QA, 5HT and 5HIAA) (Heyes, 1989) but, (see section 7.1), the integrity of the kynurenine pathway in the brain is not proven. It is possible (as suggested before) that brain 3HK is not a direct result of local tryptophan metabolism, but is instead transported from the periphery through the blood-brain barrier. Here too, the lack of correlation of brain 3HK and 3HA is perhaps providing evidence for the alternative pathway for the formation of 3HA, via the metabolism of anthranilic acid (see section

7.1). Thus, an increase in the brain concentrations may be reflecting elevated peripheral metabolism. However, the results from the preliminary study of plasma suggest that there is no significant effect here. It was speculated that if the plasma showed changes, this could have provided an important peripheral marker for the disease, that could perhaps have formed the basis of an alternative predictor of the disease. Further studies are needed to extend the number of patients in the series.

The increase of brain 3HK in HD may be reflecting changes in the activity of the enzymes involved, both synthetic and metabolic. It has been reported that kynurenine hydroxylase and kynureninase usually have low activites in rat brain. It may be hypothesized that abnormal metabolism or changes in vital cofactors could result in the elevated 3HK observed in the human brain in HD. Alternatively, an abnormal response to an influx of tryptophan could produce a consequential increase in 3HK.

The mechanism of cytotoxic damage induced by 3HK is not well characterised. A role for excitatory glutamate receptors is not yet proven, but a link with hydrogen peroxide and free radicals has been postulated (Eastman and Guilarte, 1990). Obviously further studies are needed to elucidate the mechanisms and effects in the brain, as well as a greater understanding of the metabolic factors controlling 3HK.

7.9 Vitamin B6 deficiency

From the diagram of the tryptophan metabolism (Fig 6.1) via the kynurenines, it can be seen that vitamin B6 plays a vital role, as some of the

enzymes involved have vitamin B6 dependent mechanisms. In particular kynureninase and kynurenine transaminase are sensitive to vitamin B6 deficiency, the former more so than the latter, thus a build up of particular compounds may occur. Many studies have been made to investigate the consequences of such a deficiency by measuring urinary kynurenine metabolites in various animals (Yeh and Brown, 1977; Korbitz et al., 1963; Henderson et al., 1951) and in human beings (Yess et al., 1964; Brown et al., 1965) and more recently in rat brain (Guilarte and Wagner, 1987). Predictably, the results seem to indicate that concentrations of xanthurenic acid, 3HK and kynurenine were increased in the urine of all species studied, after tryptophan loading, but changes of other metabolites varied according to the animal. Thus, urinary 3HA was not significantly changed in the rat, but increased in the guinea pig and in humans. QA concentrations were not significantly changed in the hamster and guinea pig and increased in humans (Yeh and Brown, 1977). They suggested that differential depression of the activity of the two vitamin B6 dependent enzymes occurred in each species. The data of Guilarte and Wagner (1987) showed concentrations of 3HK to be significantly increased throughout the brain in vitamin B6 deficient neonatal rats. However, the effect was not observed in adult rats, which they suggested was due to the residual vitamin B6 in these brains. The increase in brain 3HK concentrations was reversible by the injection of vitamin B6, and was accompanied by increased 5HT and 5HIAA in the frontal cortex, as well as neurological symptoms including seizures and motor excitement. The concentration of 3HK found in these neonates would appear to be within the range quoted by Eastman (1987) for neurotoxic action, thus this may be aetiologically associated with the

neuronal losses described.

Two further systems have been studied: the dopaminergic and GABAergic systems. Firstly, progeny of rats which had been fed a diet deficient in vitamin B6 had compromised dopaminergic systems, at certain stages of development. Thus, concentrations of dopamine, homovanillic acid, D2 receptor binding and activity of DOPA decarboxylase (also a vitamin B6 dependent enzyme) were all reduced in the striatum (Guilarte et al., 1987). Also present were the neurological signs of vitamin B6 deficiency: tremor, ataxia and epileptic seizures, together with weight loss. The neurochemical and neurological changes were reversible by the injection of vitamin B6. However, the neurological symptoms could be associated with other systems such as the GABAergic system, as vitamin B6 deficiency also results in the reduction of glutamate decarboxylase (another dependent enzyme). Thus, there are reduced concentrations of GABA in the vitamin B6 deficient rat brain, both in neonates and in adults (Bayoumi et al., 1972; Bayoumi and Smith, 1972). However, the adult rats do not exhibit seizures, contrary to the neonates. A postulated loss of striatal GABA neurones has also been reported in vitamin B6 deficient rats and their progeny (Wasynczuk et al., 1983). The changes described above bear some resemblance to those observed in HD, and may provide some evidence for the possibility of a dysfunction in the utilisation of vitamin B6 in HD, during development or in the adult. Alternatively, increased 3HK may be the common factor responsible for the changes described both in vitamin B6 deficiency in neonates and in HD.

A further area of interest is the investigation of hepatic encephalopathy, where there is evidence

suggesting abnormal utilisation of vitamin B6 in alcoholism (Bowman and Rand, 1984). A dysfunction of tryptophan metabolism has been implicated in hepatic encephalopathy (Freese et al., 1990) together with significantly increased QA in the cortex and CSF (Moroni et al., 1986a). Our own recent investigation showed cortical 3HK to be significantly increased in such patients (data not shown: Pearson and Reynolds, 1991). These results are consistent with an association of 3HK with vitamin B6 deficiency. The evidence that diazepam binding inhibitor is increased in CSF from patients with hepatic encephalopathy (Rothstein et al., 1989) and in the brain in HD (Ball et al., 1988) may be of consequence, as 3HK has been shown to decrease benzodiazepine receptor binding affinity in rat brain (Guilarte et al., 1987). Furthermore, Guilarte et al. suggested that the increased brain concentrations of 3HK found in neonatal vitamin B6 deficiency may be associated with seizures. More recently (Guilarte, 1991) reported reduced function of the NMDA receptor-ion channel in the brain from such rats.

Chapter 8

Conclusions

8.1 General conclusions

The work described in this thesis has identified specific changes in the concentration of neurotransmitters in specific regions of the brain in Huntington's disease (HD). This knowledge is important in the basic understanding of which neuronal populations die in HD, which survive, and why. Thus we may be able to address the challenge of preventing these changes, or in some way recompensing losses of neurotransmitters. The model of Parkinson's disease (PD) sets a precedent for successful, albeit limited, therapy (L-Dopa) following the establishment of a particular transmitter deficit. However, the paradigm is not necessarily appropriate to the study of HD, as in HD there is no single transmitter deficit, but instead there is a series of changes. The primary neuronal losses are inseparable, as yet, from the relative secondary changes, and simple GABAergic replacement therapy does not alleviate the symptomology. Following the same argument, the use of transplants of foetal tissue to replace the lost neurones, would also appear to be more likely to be effective in PD than in HD. Thus, whereas human clinical transplants have been attempted and assessed in PD, the analogy is still purely experimental in animal models of HD. Nevertheless, normal growth and functioning connections have been described from striatal grafts in the animal model of HD (Emson, 1991). One factor that may be important is that the progressive neurotoxic process will still be ongoing in the host brain and may also kill the transplanted neurones. The timing of the graft may also be a

problem as most transplant studies use acute lesions to produce animal models of HD (not comparable to advanced HD). However, the limited life expectancy and lack of adequate treatment in HD may alter the validity of its application, as compared to PD.

The study of the neurochemical or neuropathological characteristics of HD is useful, even at a time when molecular researchers predict that the gene will soon be identified and cloned. All these approaches (molecular, neurochemical and neuropathological) are relevant and should be studied concurrently, especially as the HD gene appears to be more elusive than previously anticipated. Moreover, even when the gene is identified, there may not necessarily be a clear relationship between the gene and its expressed product. This can only be adequately understood by examining all aspects of the disease process.

By the measurement of parameters such as concentrations of neurotransmitters and their metabolites, synthetic or metabolic enzyme activities, densities or affinities of receptor or uptake sites it is possible to get a profile of a neurotransmitter system. This can be compared over many regions of the brain, producing an overall view. One major consequence of neurochemical studies is the identification of subtle changes that can be specifically modulated pharmacologically; especially now that 'cleaner' drugs can be designed to affect sub-types of receptors (e.g. quipazine/5HT3, quinpirole/D3). An example of the new experimental drug therapies is the use of a glycine agonist (as an anti-choreic) to stimulate the underactive glutamatergic pathway from the subthalamic nucleus to the medial pallidum. This deficit was recently described by Penney and Young

(1983) and the studies of Crossman et al. (1988) with which our own report of abnormal pallidal γ-aminobutyric acid (GABA) balance associated with chorea was consistent. A further associated therapy to be tested is the replacement of met-enkephalin, that is contained in a pathway lost early in the disease (the striato-lateral pallidum pathway). Similarly, substance P therapy is being considered in an attempt to modify compounds localised in other striatal outputs (Chase, 1991). The strategy of modulating single pathways may be more promising than the general GABA replacement therapy which does not address the regional differences highlighted by our study. However, we found the well-established striatal deficit to extend to include all regions examined, including the cortex, where it provides evidence supporting the involvement of the cortex in HD. These GABA deficits are consistent with the neuropathological and other neurochemical markers of GABA neuronal losses. Consequently, there appears to be decreased inhibition of the nigrostriatal dopaminergic pathway.

Similarly, our study provides the first comprehensive description of decreased concentrations of glutamate throughout the brain in HD, except in the pallidum and the s.nigra, later confirmed by Ellison et al. (1987). Again, this provides evidence which is consistent with cortical changes in HD. Furthermore, these changes contradict the hypothesis that increased endogenous glutamate is causing neurotoxic degeneration. Thus neuroprotective therapies aimed at decreasing glutamatergic function may aggravate an already compromised system (e.g. MK801, kynurenic acid, glycine antagonists). Drugs aimed at increasing glutamate function (e.g. glycine agonists) may provide an alternative method of alleviating

choreiform movements. Furthermore, glutamatergic deficits are consistent with the neuropathological and other neurochemical studies implicating glutamatergic neuronal and receptor losses (e.g. N-methyl-D-aspartate (NMDA) receptors).

Our finding of indicators of reduced turnover of the dopamine (DA) system in HD in all areas measured (except the cortex) are consistent with, and extend some of the previous studies (Reynolds and Garrett, 1986; Kish et al., 1987), but provide no evidence for an increased DA system as described by others (Melamed et al., 1982; Spokes, 1980). The efficacy of dopaminergic blockers or dopamine depletors in the alleviation of the chorea in HD has been described in terms of stopping the inhibition of the dying striato-lateral pallidal met-enkephalin-containing GABA projections. Of particular interest are reports that dopaminergic agonists (e.g. apomorphine) can actually reduce choreiform movements. The mechanisms are not completely understood, but it has been suggested that presynaptic autoreceptors may be involved. The increased homovanillic acid (HVA) in the cortex has been postulated to reflect striatal degeneration and the lack of HVA change associated with neuroleptics in HD, provides evidence that an important feedback mechanism (perhaps GABAergic) is absent in HD. Other neuropathological and neurochemical evidence supports the idea of an intact nigostriatal pathway which is down-regulated resulting in reduced activity and relatively lacking in inhibition, due to the GABA and cholinergic deficits.

Another novel finding from our investigation is to extend the reduced choline acetyltransferase (CAT) activity in the striatum and hippocampus in HD, to include the frontal cortex, again indicating

cortical involvement in HD. Relatively less inhibition of the nigrostriatal dopaminergic pathway may be a consequence of such changes, although cholinergic drug therapies do not seem to be effective in the reduction of chorea.

Until our study, there were no major investigations of the 5-hydroxytryptamine (5HT) system in HD. Our results showed both 5HT and 5-hydroxyindoleacetic acid (5HIAA) to be substantially increased throughout the brain (except the hippocampus). The striatal changes were later verified by Kish et al. (1987), but they did not examine the cortex. An increase in serotonergic terminals reported previously (Cross et al., 1986b) appears consistent with our findings, and deficits of 5HT1 receptors may reflect down-regulation in response to increased 5HT function. A recent hypothesis suggests that 5HT1A receptors are on glutamatergic neurones (personal communication, Bowen, 1991); thus such a deficit (5HT1) in HD may be reflecting the cortical glutamatergic loss, described earlier (see section 3.2). Although any increases of concentrations may be relative to tissue atrophy, the substantially greater increase in 5HIAA as compared to 5HT in the temporal cortex suggests that here at least an actual increase in turnover may contribute to the results. Evidence supporting increased tryptophan function comes from our study of tryptophan metabolism, via the kynurenine pathway, which also appears to be overactive, resulting in increased concentrations of some of the metabolites in HD. We have also shown concentrations of tryptophan itself to be increased in the temporal cortex in HD, but not significantly in the striatum. Furthermore, as GABA is reportedly involved in the release of 5HT, such increases of 5HT (described above) would be predicted in association with the GABA losses in HD.

Separating a subset of the major HD group in our series, we were able to describe a previously unidentified depletion of monoamine transmitters, as a consequence of prior treatment with tetrabenazine in HD. These results confirmed animal studies demonstrating such depletions. The greatest reduction was dopamine in the caudate, which may provide the basis for the effect of tetrabenazine on the alleviation of chorea. However, monoamine losses in limbic regions may mediate the production of side effects such as depression, drowsiness and suicide. Tetrabenazine remains the drug of choice in the UK for the amelioration of choreiform movements, although it is not available for use in USA.

Of particular interest is our investigation of an asymptomatic HD case previously reported in the literature by Carrasco and Mukherji (1986). The subject had gross striatal atrophy (66% of normal control) and neuronal losses of 40% in the caudate and 80% in the putamen. As part of our study we investigated the neurochemical profile of the brain from this case, comparing the changes to controls and to our large HD series (Reynolds and Pearson, 1990a). The most striking feature was the GABA deficit observed in the caudate and lateral pallidum from the asymptomatic case. In these areas the GABA concentrations were midway between the control and HD ranges, that showed no overlap. However, in the medial pallidum there was no significant GABA loss apparent. These results indicate that degeneration of the GABAergic systems of the striatum and lateral pallidum can occur (up to a certain threshold), before the onset of the clinical symptoms of HD. The results also suggest that GABA concentrations in the medial pallidum are affected at a later stage.

Our results are consistent with a reported case by Albin et al. (1990b) describing abnormalites of NMDA receptors and the loss of substance P fibres in presymptomatic HD (with no pathological findings). Obviously further studies of these and other important presymptomatic cases may enable a greater understanding of how the disease progresses clinically, pathologically and neurochemically; this is in sharp contrast to the majority of investigations which can inevitably only examine end-stage HD.

In general the neurochemical changes described in the brain in HD are consistent with the neuropathological findings, and within the striatum the contents of the patches appear relatively spared, whereas some of the matrix markers are lost. However, a recent report suggests that within the patches there are apparent selective islands of neuronal loss, whose contents are as yet undefined (Young, 1991). Why certain neuronal populations degenerate is not known, but a further theory was postulated by Emson (1991), regarding the immunity of somatostatin/neuropeptide Y/ NADPH-diaphorase containing neurones. It was suggested that these neurones also contain nitric oxide synthetase that can form free radicals. Thus, any surrounding cells were killed off by these neurones producing free radical damage (Emson, 1991).

On another level, correlations of the symptoms of HD were examined in subgroups of the large HD series. HD provides a classic situation in which to study other diseases, as a model of neuropsychiatric disorders (including depression, schizophrenia, dementia and dyskinesia). As there is such an extensive overlap, any observations made in HD may be of potential application to these other

disorders.

In this study, we described the first neurochemical correlate of the chorea in HD. Patients with severe chorea showed a relatively smaller deficit of GABA in the medial pallidum, as compared with those having mild chorea. Thus the chorea may relate to the residual balance of GABAergic innervation between the pallidal regions. This was found to be consistent with animal models of chorea (Crossman et al., 1988) and current views of the chorea in HD (Penney and Young, 1983). A further general finding in HD was that whilst pallidal regions showed losses of GABA, the medial part shows a smaller loss than the lateral part, which is also consistent with the above reports, and other neurochemical changes (e.g. reduced glutamic acid decarboxylase activity described by Spokes, 1980). As the disease advances, the initial choreiform movements become increasingly akinetic, as the eventual degeneration of the striatal-medial pallidum pathway follows the initial loss of the lateral pallidum GABAergic innervation.

A further novel correlation comes from our study of the dementia in HD, where we were trying to provide evidence as to whether the dementia is similar to Alzheimer's disease (AD) or to PD and other subcortical dementias. Roos (1991) made a report suggesting that the plaques and tangles found in HD are only consistent with general aging and not with the dementia of the disease. AD is associated with deficits of cortical CAT activity, that correlates with cognitive impairment (Mountjoy et al., 1984). Similar deficits have been reported in PD dementia. However, in HD we showed the CAT deficit in one cortical area and the hippocampus to be unassociated with the degree of dementia. Cortical amino acids

and monoamines have also been reported to be reduced in AD (Rossor et al., 1984), but in our study there was no correlation with dementia in HD. The only neurochemical correlates of the dementia in HD were concentrations of GABA and glutamate in the caudate which had an inverse association with the severity of the dementia. This indication of the involvement of the caudate in dementia is consistent with previous PET and CAT studies in HD, in which dementia and impaired cognitive function are related to hypometabolism of the caudate and caudate atrophy (and not with meausures of cortical function or atrophy) (De la Monte et al., 1988; Bamford et al., 1989; Starkstein et al., 1988).

Further studies were intended to be made to try to subdivide the HD group to identify neurochemical correlates of other symptoms, including depression, aggression and schizophreniform psychosis. However, the numbers of these subgroups were too small for analysis. Previously described changes in these disorders in the normal population were to have been examined for their relevance to HD. Thus, in depression deficits of monoamines have been implicated (van Praag , 1977), as have cell losses in the locus coeruleus and raphé nucleus in depression in AD (Zweig et al., 1988; Iversen et al., 1983) and low CSF GABA (Lloyd et al., 1989) and enkephalin involvement (Peyser and Folstein, 1990) and increased cortico-releasing factor (CRF) in CSF (Kurlan et al., 1989). Studies looking to identify such changes in HD found no association of depression with any of the above (except for CSF CRF which was linked with the severity of major depression). The only other reports were a neuropathological investigation that linked HD depression with more severe global atrophy of the cortex and white matter (De la Monte et al., 1988),

and a report relating depression in HD with hypometabolism in the prefrontal cortex and thalamus (Peyser, 1991). Apathy in HD was found to correlate with hypometabolism of the cingulate gyrus (Peyser, 1991).

Correlates for aggression are not well characterised, although one clinical study has been described in HD (Burns et al., 1990). Mood and aggression have been suggested to be mediated by serotonergic activity (van Praag et al., 1986) and a study of aggression in AD revealed reduced concentrations of 5HT in the orbital gyrus of aggressive patients (Palmer et al., 1988).

Neurochemical correlates of schizophrenia include lateralised changes of DA concentrations in the amygdala, altered D2 receptor binding, limbic GABA uptake site deficit, increased cortical glutamatergic markers and increased cortical DA turnover (Reynolds, 1983, 1989; et al. 1990c; Deakin et al., 1989). Whether any of these changes are associated with the schizophreniform psychosis found in HD remains unproven, as there were insufficient patients to study this symptom.

A likely strategy for the management of HD involves the concept of neuroprotection, after further characterisation of the excitotoxic model of HD. Any potential neurotoxic compounds can be tested in animal models and, if a causative agent were to be identified, drugs could be developed (or existing ones used) that could prevent the progressive neuronal degeneration. It has been postulated that the aetiological agent involved in HD results from an aberrant metabolic process producing toxic concentrations. The tryptophan metabolite, quinolinic acid (QA), provides us with the best

animal model (although there are a few discrepancies) for HD. A major aim of this study was to investigate and characterise tryptophan metabolism, via the kynurenine pathway in HD. It was hoped to establish whether metabolism was normal, or producing neurotoxic concentrations of neuroactive compounds in HD. Working on the initial hypothesis for increased concentrations of QA in the brain in HD, we set out to provide evidence supporting this. Furthermore, activity of its synthetic enzyme, 3-hydroxyanthranilic acid oxygenase (3HAO), was reported to be significantly increased in the brain in HD. However, our results were the first to establish that concentrations of QA were not significantly changed in both the striatum and cortex in HD. This was later confirmed by Schwarcz (1988b), who also found no significant difference in the CSF, and they too reported the large range of values of QA concentrations (in both HD and controls) that may limit the use of such results . These findings are contrary to the working hypothesis and do not support an aetiological role for QA in HD. However, a previous transient increase can not be excluded and in vitro studies suggest that for chronic neurotoxic effects of QA, much smaller concentrations are required; thus subtle (non-significant) changes of QA concentrations may be implicated in HD.

Following these reports, a further study was made by Connick et al (1988) who postulated that although QA concentrations were unchanged in HD, a relative deficit of kynurenic acid (KA) could be causing otherwise normal levels of QA to be neurotoxic. However, they found increased concentrations of KA, in the motor cortex in HD (but not significant in the striatum) , although this change was not confirmed by the study of Beal (1990a). This

evidence for a possible dysfunction of kynurenine metabolism in HD, stimulated our hypothesis that another neuroactive kynurenine metabolite, 3-hydroxykynurenine (3HK), could be of interest in HD. Our study reported the novel finding of significantly increased concentrations of an endogenous neurotoxic compound (3HK) throughout the regions of the brain in HD. This is of particular interest as it is the first description of such an increase in HD, and it is tempting to speculate whether this compound is of aetiological significance in HD. Such an increase may merely be reflecting tissue atrophy. However, as no significant changes were observed in AD, the effect in HD appears to be associated to some degree with actual metabolic dysfunction. Furthermore, the increased cortical tryptophan concentrations in HD also provide evidence supporting an aberrant metabolic process. As tryptophan levels did not appear to correlate with 3HK, it would seem that 3HK metabolism is complex and not mediated by a simple controlling mechanism. Results from a very recent study by Beal (1990a) in the HD striatum are inconsistent with such a change in 3HK concentrations. We made a very preliminary study using blood from patients with HD to investigate the possibility of a peripheral marker for the changes of the disease. However, there appeared to be no significant difference between HD and control values (although the numbers of cases were very small) (see section 7.8).

Of note are our new results from a further study demonstrating increased concentrations of 3HK in the frontal cortex in patients with hepatic encephalopathy (Pearson and Reynolds, 1991); a dysfunction of tryptophan metabolism and impaired utilisation of vitamin B6 have previously been

implicated in this disorder. Thus, there may be some association between the changes found in both disorders and further study of 3HK metabolism is warranted in order to elucidate and characterise such tryptophan metabolism. A further point of interest is the increased diazepam binding inhibitor in HD brain and in cerebrospinal fluid from patients with hepatic encephalopathy. Whether this has any relevance to the decreased benzodiazepine receptor binding effect of 3HK remains to be seen.

These studies have demanded the development and adaptation of new methods in order to determine the concentrations of neurotransmitters, amino acids and other neuroactive compounds, as well as the activities of enzymes. Specifically, novel methods have been worked up (and published) for the measurement of concentrations of QA, and for 3HK, and for the amino acids.

8.1 Further work

To increase the numbers of cases in order to extend the neurochemical correlates of the symptoms of the disease; in particular, to include depression, aggression and schizophreniform psychosis.

To characterise NMDA receptor binding and associated sites, in HD to identify any abnormalities.

To extend the regional distribution of 3HK in the brain in controls and in HD, including less atrophied areas. This would enable us to understand better whether the changes in HD are global. Furthermore, extending the peripheral measurement of 3HK in HD (by increasing the number of cases), will provide real evidence as to whether the effects observed in the brain have a marker in the periphery

and whether they are related.

To examine the in vitro effects of 3HK in cerebellar cultures in order to characterise the mode of action of the neurotoxic damage. In particular, to identify whether the effects are mediated via the NMDA, AMPA or other receptors, by the use of appropriate antagonists, and comparison of the effects with those of known agonists.

To compare the consequences of intra-striatal injection of 3HK in the rat with established animal models for HD (such as QA). This would involve the comparison of neurochemical and neuropathological changes evoked by 3HK with those seen in HD and its animal models.

To study the enzymes involved in the metabolism of tryptophan via the kynurenine pathway in the brain, both in controls and in HD. The aim is to establish any controlling enzymes, co-factors or feedback mechanisms which could influence the build up of 3HK, or other neuroactive compounds in the pathway. Also, to examine the effects of precursor loading (such as tryptophan or kynurenine) to establish whether the kynurenine pathway actually functions in the human brain. Finally, to identify any specific differences occurring in the HD brain as compared to control function.

To study benzodiazepine receptor binding in the brain in HD, particularly to examine the effects of 3HK at this site (and in hepatic encephalopathy).

References

Abdul-Fadl MAM and Khalafallah AS (1961) Studies on the urinary excretion of certain tryptophan metabolites in bilharziasis and its possible relation to bladder cancer in Egypt. Brit J Cancer 15, 479-482

Albert ML, Feldman RG, Willis AL (1974) The 'subcortical dementia' of supranuclear palsy. J Neurol Neurosurg Psychiat 37, 121-130

Albin RA, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12, 366-375

Albin RL, Reiner A, Anderson KD et al (1990a) Striatal and nigral neuron subpopulations in rigid Huntington's disease: implications for the functional anatomy of chorea and rigidity-akinesia. Ann Neurol 27, 357-365

Albin RL, Young AB, Penney JB et al (1990b) Abnormalities of striatal projection neurons and N-methyl-D-aspartate receptors in presymptomatic Huntington's disease. N Eng J Med 322, 1293-1298

Allenmark S, Bengtsson M-B, Nordstrom K et al (1984) Determination of endogenous 3-hydroxykynurenine in plasma. Microchem J 29, 106-112

Allison LA, Mayer GS, Soup RE (1984) O-phthalaldehyde derivatives of amines for high speed liquid chromatography. Anal Chem 56, 1089-1096

Aprison MH, Davidoff RA, Werman R (1970) Glycine: Its metabolic and possible transmitter roles in nervous tissue. In: Handbook of Neurochemistry, 3 (ed. Lajtha A) Plenum, New York

Aquilonius S-M, Eckernas S-A, Sundwall A (1975) Regional distribution of choline acetyltransferase in the human brain: changes in Huntington's chorea. J Neurol Neurosurg Psychiat 38, 669-677

Aquilonius S-M, Eckernas S-A (1977) Choline therapy in Huntington's chorea. Neurol 27, 887-889

Arai H, Emson PC, Carrasco LH (1987) Huntington's disease: changes in tachykinin content in postmortem brains. Ann Neurol 22, 587-594

Aronin N, Cooper PE, Lorenz LJ et al (1983) Somatostatin is increased in the basal ganglia in Huntington's disease. Ann Neurol 13, 519-26

Arregui A, Emson PC, Spokes EG (1978) Angiotensin-converting enzyme in substantia nigra: Reduction of

activity in Huntington's disease and after intrastriatal kainic acid in rats. Eur J Pharmacol 52, 121-124

Ball MJ, Hachinski V, Fox A et al (1985) A new definition of Alzheimer's disease: A hippocampal dementia. Lancet i, 14-16

Ball JA, Burnet PWJ, Bretherton-Watt D et al (1988) Diazepam-inhibitor in suicide, Alzheimer's dementia and Huntington's disease. Neurosci Lett Suppl 32, S56

Bamford KA, Caine ED, Kido DK et al(1989) Clinical-pathological correlation in Huntington's disease: a neuropsychological and computed tomography study. Neurol 39, 796-801

Baran H, Schwarcz R (1990) Presence of 3-hydroxyanthranilic acid in rat tissues and evidence for its production from anthranilic acid in the brain. J Neurochem 55, 738-744

Barbeau A (1970) Parental ascent in the juvenile form of Huntington's chorea. Lancet ii, 937

Baskin SI, Leibman AJ, Cohen EM (1976) Possible functions of taurine in the central nervous system. Adv Biochem Psychpharmacol 115, 153-164

Battie C, Verity MA (1981) Presence of kynurenine hydroxylase in developing rat brain. J Neurochem 36, 1308-1310

Bayoumi RA, Kirwan JR, Smith WRD (1972) Some effects of dietary vitamin B6 deficiency and 4-deoxypyridoxine on γ-aminobutyric acid metabolism in rat brain. J Neurochem 19, 569-576

Bayoumi RA, Smith WRD (1972) Some effects of dietary vitamin B6 on γ-aminobutyric acid in developing rat brain. J Neurochem 19, 1883-1897

Beal MF, Kowall NW, Ellison DW et al (1986) Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. Nature 321, 168-171

Beal MF, Kowall NW, Martin JB (1987) No evidence for preservation of somatostatin-containing neurons after intrastriatal injections of quinolinic acid: reply. Nature 327, 329

Beal MF, Ellison DW, Mazurek MF et al (1988a) A detailed examination of substance P in pathologically graded cases of Huntington's disease. J Neurol Sci 84, 51-61

Beal MF, Kowall NW, Swartz KJ et al (1988b) Model of Huntington's disease: response. Science 241, 475

Beal MF, Mazurek MF, Ellison DW et al (1988c) Somatostatin and neuropeptide Y concentrations in pathologically graded cases of Huntington's disease. Ann Neurol 23, 562-569

Beal MF, Swartz KJ, Finn SF et al (1988d) Amino acid and neuropeptide neurotransmitters in Huntington's disease cerebellum. Brain Res 454, 393-396

Beal MF, Matson WR, Swartz KJ et al (1990a) Kynurenine pathway measurements in Huntington's disease striatum: evidence for reduced formation of kynurenic acid. J Neurochem 55, 1327-1339

Beal MF, Kowall NW, Swartz KJ et al (1990b) Homocysteic acid lesions in rat striatum spare somatostatin-neuropeptide Y (NADPH-diaphorase) neurones. Neurosci Lett 108, 36-42

Beal MF, Ferrante RJ, Swartz KJ et al (1991a) Chronic quinolinic acid lesions in rats closely resemble Huntington's disease. J Neurosci 11, 1649-1659

Beal MF, Swartz KJ, Finn SF et al (1991b) Neurochemical characterisation of excitotoxin lesions in the cerebral cortex. J Neurosci 11, 147-158

Beaumont K, Maurin Y, Reisine TD et al (1979) Huntington's disease and it's animal model: alterations in kainic acid binding. Life Sci 24, 809-816

Belenduik K, Belenduik GW, Freedman DX (1979) Platelet serotonin and platelet MAO activity in individuals with Huntington's disease. Adv Neurol 23, 473-480

Beninger RJ, Jhamandas K, Boegman RJ et al (1986) Kynurenic acid-induced protection of neurochemical and behavioural deficits produced by quinolinic acid injections into the nucleus basalis of rats. Neurosci Lett 68, 317-321

Berent S, Giordani B, Lehteinen S et al (1988) Positron emission tomographic scan investigations of Huntington's disease: cerebral metabolic correlates of cognitive function. Ann Neurol 23, 541-546

Bernheimer H, Hornykiewicz O (1973) Brain amines in Huntington's chorea. Adv Neurol 1, 525-531

Bird ED (1974) A sex-related factor in the inheritance of Huntington's chorea. Ann Hum Genet

37, 255-260

Bird ED, Iversen LL (1974) Post-mortem measurement of glutamic acid decarboxylase, choline acetyltransferase and dopamine in basal ganglia. Brain 97, 457-472

Bird ED, Chiappa SA, Fink G (1976) Brain immunoreactive gonadotrophin-releasing hormone in Huntington's chorea and in non-choreic subjects. Nature 260, 536-538

Bird ED, Gale JS, Spokes EGS (1977) Huntington's chorea: Post-mortem activity of enzymes involved in cerebral glucose metabolism. J Neurochem 29, 539-545

Bird ED (1980) Chemical pathology of Huntington's disease. Ann Rev Pharmacol Toxicol 20, 533-551

Bird ED, Spokes EGS (1982) Huntington's chorea. In: Disorders of Neurohumoural Transmission (ed. Crow TJ), Academic Press Inc, London

Bird ED, Coyle JT (1986) Huntington's disease. In: Clinical Neurochemistry Vol 1 (eds. Bachelard HS, Lunt GG, Marsden CD) Academic Press Inc, London

Blundell JE (1984) Serotonin and appetite. Neuropharmacol 23, 1537-1551

Boegman RJ, El-Defrawy SR, Jhamandas K et al (1985) Quinolinic acid neurotoxicity in the nucleus basalis antagonised by kynurenic acid. Neurobiol Aging 6, 331-336

Boegman RJ, Rolfe BT, Riopelle RJ et al (1986) Dipicolinic acid protects against quinolinic acid-induced cell death. Soc Neurosci Abstract 92.6

Boegman RJ, Smith Y, Parent A (1987) Quinolinic acid does not spare striatal neuropeptide Y-immunoreactive neurons. Brain Res 415, 178-182

Bonilla E, Prasad ALN, Arrieta A (1988) Huntington's disease: studies on brain free amino acids. Life Sci 42, 1153-1158

Borri PF, Opdenvelde WM, Hooghwinkel GJM et al (1967) Biochemical studies in Huntington's chorea. VI. Composition of striatal neutral lipids, phospholipids, glycolipids, fatty acids and amino acids. Neurol 17, 172-178

Bowen DM, Allen SJ, Benton JS et al (1983) Biochemical assessment of serotonergic and cholinergic dysfunction and cerebral atrophy in Alzheimer's disease. J Neurochem 41, 266-272

Bowers MB, Hoffman FJ (1986) Homovanillic acid in caudate and prefrontal cortex following acute and chronic neuroleptic administration. Psychopharmacol 88, 63-65

Bowman WC, Rand MJ (1984) Textbook of Pharmacology, Blackwell Scientific Press, Oxford

Brown RR, Yess N, Price JM et al (1965) Vitamin B6 depletion in man: Urinary excretion of quinoinic acid and niacin metabolites. J Nutr 87, 419-423

Brown WT, Sanberg PR, McGeer PL (1979) Corticosteroids and chorea. Arch Neurol 7, 452-453

Bruyn GW, Bots GTAM, Dom R (1979) Huntington's chorea: current neuropathological status. Adv Neurol 23, 83-93

Bruyn RPM, Stoof JC (1990) The quinolinic acid hypothesis in Huntington's chorea. J Neurol Sci 95, 29-38

Burke RE, Reches A, Traub MM, et al (1985) Tetrabenazine induces acute dystonic reactions. Ann Neurol 17, 200-202

Burns A, Folstein S, Brandt J et al (1990) Clinical assessment of irritability, aggression and apathy in Huntington and Alzheimer disease. J Nerv Ment Dis 178, 20-25

Butterfield DA, Markesbery WR (1981) Huntington's disease: a generalised membrane defect. Life Sci 28, 1117-1131

Butterworth J, Yates CM, Reynolds GP (1985) Distribution of phosphate-activated glutaminase, succinic dehydrogenase and é-glutamyl transpeptidase in post-mortem brain from Huntington's disease and agonal cases. J Neurol Sci 67, 161-171

Butterworth J (1986) Changes in nine enzyme markers for neurons, glia, and endothelial cells in agonal state and Huntington's disease caudate nucleus. J Neurochem 47, 583-587

Caraceni T, Calderini G, Consolazione A et al (1977) Biochemical aspects of Huntington's chorea. J Neurol Neurosurg Psychiatr 40, 581-587

Carlsson A (1976) Some aspects of dopamine in the basal ganglia. In: The Basal Ganglia, 181-189 (ed. Yahr MD) Raven Press, New York

Carrasco LH, Mukherji CS (1986) Atrophy of corpus striatum in normal male at risk of Huntington's

chorea. Lancet i, 1388-1389

Carter CJ (1981) Loss of glutamine synthetase activity in the brain in Huntington's disease. Lancet i, 782-783

Carter CJ (1984) Reduced GABA transaminase activity in the Huntington's disease putamen. Neurosci Lett 48, 339-342

Chandler JL and Gholson RK (1972) Studies on the de novo biosynthesis of NAD in Esherichia coli: Quantitative method for isolating quinolinic acid from biological materials. Anal Biochem 48, 529-535

Chase TN (1973) Biochemical and pharmacologic study of monoamines in Huntington's chorea. Adv Neurol 1, 533-542

Chase TN (1991) Abstract: World Fed Neurol Research Group on Huntington's chorea

Chaves-Carballo E (1987) Rett's syndrome: Urine organic acid abnormalities. Ann Neurol 22, 442

Chesis Pl, Levin DE, Smith MT et al (1984) Mutagenicity of quinones: Pathways of metabolic activation and detoxification. Proc Natl Acad Sci 81, 1696-1700

Choi DW (1990) Glutamate receptor-mediated injury in stroke. Meeting Proceedings of "Neurodegeneration and neuroprotection"

Chui E (1979) Notes on the management of Huntington's disease. Aus Fam Physician 8, 197-200

Chui HC (1989) Dementia. A review emphasizing clinicopathologic correlation and brain-behaviour relationships. Arch Neurol 46, 806-814

Connick JH, Lombardi G, Beni M et al (1988) Decrease in rat cerebral quinolinic acid concentration following chronic hydrocortisone treatment. Neurosci Lett 88, 216-220

Connick JH, Carla V, Moroni F et al (1989) Increase in kynurenic acid in Huntington's disease cortex. J Neurochem 52, 985-987

Connick JH, Stone TW (1989) Quinolinic acid neurotoxicity: protection by intracerebral phenylisopropyladenosine (PIA) and potentiation by hypotension. Neurosci Lett 101, 191-196

Cooper JR, Bloom FE, Roth RH (1982) The Biochemical Basis of Neuropharmacology, Oxford University Press, New York

Courville CB, Nusbaum RE, Butt EM (1963) Changes in trace metals in brain in Huntington's chorea. Arch Neurol 8, 481-489

Coyle JT, Schwarcz R (1976) Lesions of striatal neurones with kainic acid provides a model for Huntington's chorea. Nature 263, 244-246

Crawford MA, Hansen IL, Lopez A (1972) The excretion of 3-hydroxyanthranilic acid and quinolinic acid in Uganda africans. Brit J Cancer 23, 644-654

Cross AJ, Waddington JL (1981) Substantia nigra gamma-aminobutyric acid receptors in Huntington's disease. J Neurochem 37, 321-324

Cross AJ, Rosssor M (1983) Dopamine D1 and D2 receptors in Huntington's disease. Eur J Pharmacol 88, 223-229

Cross AJ, Crow TJ, Dawson JM et al (1986a) Subcellular pathology of human neurodegenerative disorders: Alzheimer-type dementia and Huntington's disease. J Neurochem 47, 882-889

Cross AJ, Reynolds, Hewitt LM et al (1986b) Brain serotonin receptors in Huntington's disease. Neurochem Int 9, 431-436

Cross AJ, Slater P, Reynolds GP (1986c) Reduced high affinity glutamate uptake sites in Huntington's disease. Neurosci Lett 67, 198-202

Crossman AR, Sambrook MA, Jackson A (1984) Experimental hemichorea/ hemiballismus in the monkey. Studies on the intracerebral site of action in a drug-induced dyskinesia. Brain 107, 579-596

Crossman AR (1987) Primate models of dyskinesia: the experimental approach to the study of basal-ganglia related involuntary movement disorders. Neurosci 21, 1-40

Crossman AR, Mitchell IJ, Sambrook MA et al (1988) Chorea and myoclonus in the monkey induced by gamma-aminobutyric acid antagonism in the lentiform complex. The site of drug action and a hypothesis for the neural mechanisms of chorea. Brain 111, 1211-1233

Crow TJ, Baker HF, Cross AJ (1979) Monoamine mechanisms in chronic schizophrenia: Post-mortem neurochemical findings. B J Psychiatr 134, 249-256

Cudkowicz M, Kowall NW (1990) Degeneration of pyramidal projection neurons in Huntington's disease cortex. Ann Neurol 27, 200-204

Cummings JL, Benson DF (1984) Subcortical dementia: Review of an emerging concept. Arch Neurol 41, 874-879

Czuczwar SJ, Meldrum BS (1982) Protection against chemically induced seizures by 2-amino-7-phosphono-heptanoic acid. Eur J Pharmac 83, 335-338

Czudek C, Reynolds GP (1990) (3H)Nipecotic acid binding to γ-aminobutyric aicd uptake sites in postmortem human brain. J Neurochem 55, 165-168

Danycsz W, Fadda E, Wroblewski JT et al (1984) Kynurenate and 2-amino-5-phosphonovalerate interact with multiple binding sites of the N-methyl-D-aspartate-sensitive glutamate receptor domain. Neurosci Lett 96, 340-344

Davenport CJ, Monyer H, Choi DW (1988) Tetrahydroaminoacridine selectively attenuates NMDA receptor-mediated neurotoxicity. E J Pharmacol 154, 73-78

Davies SW, Roberts PJ (1987) No evidence for preservation of somatostatin-containing neurons after intrastriatal injections of quinolinic acid. Nature, 327, 326 -329

Davies SW (1988) Model of Huntington's disease. Science 241, 474-475

Davies SW, Roberts PJ (1988) Sparing of cholinergic neurons following quinolinic acid lesions of the rat striatum. Neurosci 26, 387-393

Dawbarn D, DeQuidt ME, Emson PC (1985) Survival of basal ganglia neuropeptide-Y somatostatin neurons in Huntington's disease. Brain Res 340, 251-260

Dawbarn D, Zamir N, Waters CM et al (1986) Peptides derived from prodynorphin are decreased in basal ganglia of Huntington's disease brain. Brain Res 373, 155-158

Deakin JFW, Slater P, Simpson MDC et al (1989) Frontal cortical and left temporal glutamatergic dysfunction in schizophrenia. J Neurochem 52, 1781-1786

De Keyser J, De Backer J-P, Ebinger G et al (1989a) Coupling of D1 dopamine receptors to the guanine nucleotide binding protein Gs is deficient in Huntington's disease. Brain Res 496, 327-330

De Keyser J, Roos RAC, Ebinger G et al (1989b) Lack of GTP-insensitive D2 dopamine receptors in Huntington's disease. J Neurol Sci 92, 329-335

De la Monte SM, Vonsattel JP, Richardson EP (1988) Morphometric demonstration of atrophic changes in the cerebral cortex, white matter, and neostriatum in Huntington's disease. J Neuropath Exp Neurol 47, 516-525

Dewey RB, Jankovic J (1989) Hemiballism-hemichorea. Clinical and pharmacological findings in 21 patients. Arch Neurol 46, 862-867

Diamond BI, Borison RL (1978) Enkephalins and nigrostriatal function. Neurol 28, 1085-1088

Dose M (1991) Abstract: World Fed Neurol Research Group on Huntington's chorea

Du F, Okuno E, Whetsell WO et al (1990) Distribution of quinolinic acid phosphoribosyltransferase in the human hippocampal formation and parahippocampal gyrus. J Comp Neurol 295, 71-82

During MJ, Freese A, Heyes MP et al (1989a) Neuroactive metabolites of L-tryptophan, serotonin and quinolinic acid, in striatal extracellular fluid. Effect of tryptophan loading. FEBS 247, 438-444

During MJ, Heyes MP, Freese A et al (1989b) Quinolinic acid concentrations in striatal extracellular fluid reach potentially neurotoxic levels following systemic L-tryptophan loading. Brain Res 476, 384-387

Eastman CL, Guilarte TR (1989) Cytotoxicity of 3-hydroxykynurenine in a neuronal hybrid cell line. Brain Res 495, 225-231

Eastman CL, Guilarte TR (1990) The role of hydrogen peroxide in the in vitro cytotoxicity of 3-hydroxykynurenine. Neurochem Res 15, 1101-1107

Ellison DW, Beal MF, Mazurek MF et al (1987) Amino acid neurotransmitter abnormalities in Huntington's disease and the quinolinic acid model of Huntington's disease. Brain 110, 1657-1673

Emson PC (1986) Neuropeptides and the pathology of Huntington's disease. Progress in Brain Research 66, 91-105

Emson PC (1991) Abstract: World Fed Neurol Research Group on Huntington's chorea

Enna SJ, Bennett P, Bylund DB et al (1976a) Alterations of brain neurotransmitter receptor binding in Huntington's chorea. Brain Res 116, 531-537

Enna SJ, Bird ED, Bennett JP et al (1976b) Huntington's chorea: changes in neurotransmitter receptors in the brain. New Eng J Med 294, 1305-1309

Fahn S (1979) Treatment of choreic movements with perphenazine. Life Sci 24, 1727-1738

Feldblum S, Rougier A, Loiseau H et al (1988) Quinolinic acid-phosphoribosyl transferase activity is decreased in epileptic human brain tissue. Epilepsia 29, 523-529

Ferrante RJ, Beal MF, Kowall NW et al (1987a) Sparing of acetylcholinesterase-containing striatal neurons in Huntington's disease. Brain Res 411, 162-166

Ferrante RJ, Kowall NW (1987) Tyrosine hydroxylase-like immunoreactivity is distributed in the matrix compartment of normal human and Huntington's disease striaum. Brain Res 416, 141-146

Ferrante RJ, Kowall NW, Beal MF et al (1987b) Morphologic and histochemical characteristics of a spared subset of striatal neurons in Huntington's disease. J Neuropath Exp Neurol 46, 12-27

Ferrante RJ, Kowall NW, Gerfen CR et al (1988) Immunocytochemical localisation of calcium-binding protein in normal and Huntington's disease striatum. J Neuropath Exp Neurol 47, 352

Ferrante RJ, Kowall NW, Richardson EP (1989) Neuronal and neuropil loss in the substantia nigra in Huntington's disease. J Neuropath Exp Neurol 48, 380

Ferriero DM, Arcavi LJ, Sagar SM et al (1988) Selective sparing of NADPH-diaphorase neurons in neonatal hpoxia-ischaemia. Ann Neurol 24, 670-676

Filloux F, Wagster MV, Folstein S (1990) Nigral dopamine type-1 receptors are reduced in Huntington's disease: a postmortem autoradiographic study using (3H) SCH 23390 and correlation with (3H) forskolin binding. Exp Neurol 110, 219-227

Fog R, Pakkenberg H (1970) Combined Nitoman treatment of Huntington's chorea and other hyperkinetic syndromes. Acta Neurol Scand 46, 249-251

Fonnum F (1975) A rapid radiochemical method for the determination of choline acetyltransferase. J Neurochem 24, 407-409

Fonnum F, Storm-Mathisen J, Divac I (1981) Biochemical evidence for glutamate as neurotransmitter in corticostriatal and corticothalamic fibres in rat brain. Neurosci 6, 863-873

Fonnum F (1984) Glutamate: a neurotransmitter in mammalian brain. J Neurochem 42, 1-11

Foster AC, Miller LP, Oldendorf WH et al (1984a) Studies of the disposition of quinolinic acid after intracerebral or systemic administration in the rat. Exp Neurol 84, 428-440

Foster AC, Schwarcz R (1985) Characterisation of quinolinic acid phosphoribosyltransferase in human blood and observations in Huntington's disease. J Neurochem 45, 199-205

Foster AC, Whetsell WO, Bird ED et al (1985) Quinolinic acid phosphoribosyltransferase in human and rat brain: activity in Huntington's disease and in quinolinate-lesioned rat striatum. Brain Res 336, 207-214

Foster AC, Okuno E, Brougher DS et al (1986a) A radioenzymatic assay for quinolinic acid. Anal Biochem 158, 98-103

Foster AC, Gill R, Iversen LL et al (1987) Systemic administration of MK-801 protects against ischaemia-induced hippocampal neurodegeneration in the gerbil. B J Pharmacol 90, 9P

Foster AC, Kemp JA (1989) Glycine maintains excitement. Nature 338, 377-378

Foster AJ, Collins JF, Schwarcz R (1983) On the excitotoxic properties of quinolinic acid, 2,3-piperidine dicarboxylic acids and structurally related compounds. Neuropharmacol 22, 1331-1342

Foster AJ, Vezzani A, French D et al (1984b) Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. Neurosci Lett 48, 273-278

Foster AJ, White RJ, Schwarcz R (1986b) Synthesis of quinolinic acid by 3-hydroxyanthranilic acid oxygenase in rat brain tissue in vitro. J Neurochem 47, 23-30

Foster JB (1986) Subcortical dementia. B Med J 292, 1035-1036

Frattola L, Albiazzati MG, Spano PF et al (1977) Treatment of Huntington's chorea with bromocryptine. Acta Neurol Scand 56, 37-45

Freese A, Swartz KJ, During MJ et al (1990) Kynurenine metabolites of tryptophan: implications for neurologic diseases. Neurol 40, 691-695

Fukui S, Schwarcz R, Rapoport SL et al (1991) Blood-brain barrier transport of kynurenines: implications for brain synthesis and metabolism. J Neurochem 56, 2007-2017

Fuller NJ, Stirling DM, Dunnett S et al (1987) Decreased brown adipose tissue thermogenic activity following a reduction in brain serotonin by intraventricular p-chloropheylalanine. Biosci Rep 7, 121-127

Gal EM (1974) Cerebral tryptophan-2,3-dioxygenase (pyrrolase) and its induction in rat brain. J Neurochem 22, 861-863

Gal EM, Sherman AD (1978) Synthesis and metabolism of L-kynurenine in rat brain. J Neurochem 30, 607-613

Gal EM, Sherman AD (1980) L-kynurenine: Its synthesis and possible regulatory function in brain. Neurochem Res 5, 223-229

Gale JS, Bird ED, Spokes EGS et al (1978) Human brain substance P: Distribution in controls and Huntington's disease. J Neurochem 30, 633-634

Ganong AH, Cotman CW (1986) Kynurenic acid and quinolinic acid act at N-methyl-D-aspartate receptors in the rat hippocampus. J Pharmacol Exp Therap 236, 293-299

Garthwaite G, Garthwaite J (1987) Quinolinate mimics neurotoxic actions of N-methyl-D-aspartate in rat cerebellar slices. Neurosci Lett 79, 35-39

Garthwaite J (1990) Mechanisms of excitatory amino acid-induced degeneration. Meeting Proceedings of "Neurodegeneration and neuroprotection"

Germano IM, Pitts LH, Meldrum BS et al (1987) Kynurenate inhibition of cell excitation decreases stroke size and deficits. Ann Neurol 22, 730-734

Gerson SC, Baldessarini RJ (1980) Motor effects of serotonin in the central nervous system. Life Sci 27, 1435-1451

Godefroy F, Matson WR, Gamache PH et al (1990) Simultaneous measurements of tryptophan and its metabolites, kynurenine and serotonin, in the superficial layers of the spinal dorsal horn. A study in normal and arthritic rats. Brain Res 526,

169-172

Goebel HH, Heipertz R, Scholz W et al (1978) Juvenile Huntington's chorea: Clinical, ultrastructural and biochemical studies. Neurol 28, 23-31

Goldblatt J, Bryer A (1987) Huntington's disease: deterioration in clinical state during treatment with angiotensin converting enzyme inhibitor. B Med J 294, 1659-1660

Goodman SI, Norenberg MD, Shikes Rh et al (1977) Glutaric aciduria: Biochemical and morphologic considerations. J Ped 90, 746-750

Goto S, Hirano A, Rojas-Corona RR (1989) Immunohistochemical visualisation of afferent nerve terminals in human globus pallidus and its alteration in neostriatal neurodegenerative disorders. Acta Neuropath 78, 543-550

Goto S, Hirano A (1990) Synaptophysin expression in the striatum in Huntington's disease. Acta Neuropath 80, 88-91

Gottfries CG, Roos BE, Winblad B (1974) Determination of 5-hydroxytryptamine, 5-hydroxyindoleacetic acid and homovanillic acid in brain tissue from an autopsy material. Acta Psychiatr Scand 50, 496-507

Grafton ST, Mazziotta JC, Pahl JJ et al (1990) A comparison of neurologic, metabolic, structural and genetic evaluations in persons at risk for Huntington's disease. Ann Neurol 28, 614-621

Gramsbergen JB, Veenma-Van der Kuin L, Venema K et al (1986) Cerebral cation shifts and amino acids in Huntington's disease. Arch Neurol 43, 1276-1281

Graveland GA, Williams RS, DiFiglia M (1985) Evidence for degenerative and regenerative changes in neostriatal spiny neurons in Huntington's disease. Science 227, 770-773

Gray PN, May PC, Mundy L et al (1980) L-glutamate toxicity in Huntington's disease fibroblasts. Biochem Biophysic Res Comm 95, 707-714

Graybiel AM, Ragsdale CW (1978) Histochemically distinct compartments in the striatum of human, monkey, and cat demonstrated by acetylthiocholinesterase staining. Proc Natl Acad Sci 75, 5723-5726

Graybiel AM (1984) Correspondence between the dopamine islands and striosomes of the mammalian

striatum. Neurosci 13, 1157-1187

Greenamyre JT, Penney JB, Young AB et al (1984) Alterations in L-glutamate binding in Alzheimer's and Huntington's diseases. Science 227, 1496-1499

Guilarte TR, Wagner HN (1987) Increased concentrations of 3-hydroxykynurenine in vitamin B6 deficient neonatal rat brain. J Neurochem 49, 1918-1926

Guilarte TR, Wagner HN, Frost JJ (1987) Effects of perinatal vitamin B6 deficency on dopaminergic neurochemistry. J Neurochem 48, 432-439

Guilarte TR, Block LD, Wagner HN (1988) The putative endogenous convulsant 3-hydroxykynurenine decreases benzodiazepine receptor binding affinity: Implications to seizures associated with neonatal vitamin B6 deficiency. Pharmacol Biochem Behav 30, 665-668

Guilarte TR (1991) Reduced NMDA receptor-ion channel function in the vitamin B-6 restricted neonatal rat brain. Neurosci Lett 121, 207-210

Gusella JF, Wexler NS, Conneally M et al (1983) A polymorphic DNA marker genetically linked to Huntington's disease. Nature 306, 234-238

Hallervorden J (1957) Huntingtonsche Chorea (Chorea chronica progressiva hereditaria)-Chorea minor, Chorea gravidarum, Senile chorea, und andere Choreaformen. In: Handbuch der speziellen pathologischen Anatomie und Histologie Part 1a, 13, 793-835 (eds. Lubarsch O, Henke H, Rossle R) Springer-Verlag, Berlin

Hammond P, Brimijoin S (1988) Acetylcholinesterase in Huntington's and Alzheimer's diseases: simultaneous enzyme assay and immunoassay of multiple brain regions. J Neurochem 50, 1111-1116

Hashimoto T, Yanagisawa N (1990) Acute reduction and long-term improvement of chorea with ceruletide (cholecystokinin analogue). J Neurol Sci 100, 178-185

Hayden MR, Vinik AI, Paul M et al (1977) Impaired prolactin release in Huntington's chorea. Evidence for a dopaminergic excess. Lancet ii, 423-426

Hayden MR (1981) Huntington's chorea. Springer-Verlag, Berlin

Hays SE, Goodwin FK, Paul SM (1981) Cholecystokinin receptors are decreased in basal ganglia and cerebral cortex of Huntington's disease. Brain Res

225, 452-456

Heeley AF, McCubbing DG, Sheperd J (1966) Effect of pyridoxine on the metabolism of tryptophan and branched-chain amino acids in two mentally retarded sibs. Arch Dis Childh 41, 652-657

Henderson LM, Hirsch HM (1949) Urinary excretion by the rat following tryptophan and 3-hydroxyanthranilic acid administration. J Biol Chem 181, 667-675

Henderson LM, Weinstock IM, Ramasarma GB (1951) Effect of deficiency of B vitamins on the metabolism of tryptophan by the rat. J Biol Chem 189, 19-29

Henn FA, Hamberger A (1971) Glial cell function: Uptake of transmitter substances. Proc Nat Acad Sci 68, 2686-2690

Heyes MP, Garnet ES, Brown RR (1985) Normal excretion of quinolinic acid in Huntington's disease. Life Sci 37, 1811-1816

Heyes MP (1987) Hypothesis: a role for quinolinic acid in the neuropathology of glutaric aciduria type I. Can J Neurol Sci 14, 441-443

Heyes MP (1988) Quantification of 3-hydroxykynurenine in brain by high-performance liquid chromatography and electrochemical detection. J Chromatog 428, 340-344

Heyes MP, Kim P, Markey SP (1988) Systemic lipopolysaccharide and pokeweed mitogen increase quinolinic acid content of mouse cerebral cortex. J Neurochem 51, 1946-1948

Heyes MP, Markey SP (1988a) (18 O)Quinolinic acid: its esterification without back exchange for use as internal standard in the quantification of brain and CSF quinolinic acid. Biomed Enviro Mass Spect 15, 291-293

Heyes MP, Markey SP (1988b) Quantification of quinolinic acid in rat brain, whole blood, and plasma by gas chromatography and negative chemical ionisation mass spectrometry: effects of systemic L-tryptophan administration on brain and blood quinolinic acid concentrations. Anal Biochem 174, 349-359

Heyes MP, Quearry BJ, Markey SP (1989a) Systemic endotoxin increases L-tryptophan, 5-hydroxyindoleacetic acid, 3-hydroxykynurenine and quinolinic acid content of mouse cerebral cortex. Brain Res 491, 173-179

Heyes MP, Rubinow D, Lane C et al (1989b) Cerebrospinal fluid quinolinic acid concentrations are increased in acquired immune deficiency syndrome. Ann Neurol 26, 275-277

Heyes MP, Gravell M, London WT et al (1990a) Sustained increases in cerebrospinal fluid quinolinic acid concentrations in rhesus macaques (Macca mulatta) naturally infected with simian retrovirus type-D. Brain Res 531, 148-158

Heyes MP, Lackner A (1990) Increased cerebrospinal fluid quinolinic acid, kynurenic acid in acute septicemia. J Neurochem 55, 338-341

Heyes MP, Mefford IN, Quearry BJ et al (1990b) Increased ratio of quinolinic acid to kynurenic acid in cerebrospinal fluid of D retrovirus-infected rhesus macaques: relationship to clinical and viral status. Ann Neurol 27, 666-675

Heyes MP, Papagapiou M, Leonard C et al (1990c) Brain and plasma quinolinic acid in profound insulin-induced hypoglycemia. J Neurochem 54, 1027-1033

Heyes MP, Brew BJ, Martin A et al (1991a) Quinolinic acid in cerebrospinal fluid and serum in HIV-1 infection: relationship to clinical and neurological status. Ann Neurol 29, 202-209

Heyes MP, Swartz KJ, Markey SP et al (1991b) Regional brain and cerebrospinal fluid quinolinic acid concentrations in Huntington's disease. Neurosci Lett 122, 265-269

Hiley CR, Bird ED (1974) Decreased muscarinic receptor concentration in post-mortem brain in Huntington's chorea. Brain Res 80, 355-358

Hoes MJAJM, Loeffen T, Vree TB (1981) Kinetics of L-tryptophan in depressive patients: a possible correlation between the plasma concentrations of L-tryptophan and some psychiatric rating scales. Psychopharmacol 75, 350-353

Hoes MJAJM, Sijben N (1981) The clinical significance if disordered renal excretion of xanthurenic acid in depressive patients. Psychopharmacol 75, 346-349

Hoffman PL, Rabe CS, Moses F et al (1989) N-methyl-D-aspartate receptors and ethanol: Inhibition of calcium flux and cyclic GMP production. J Neurochem 52, 1937-1940

Hokfelt R, Rehfeld JF, Skirboll L et al (1980) Evidence for coexistence of dopamine and CCK in

mesolimbic neurons. Nature 285, 476-478

Hokfelt T, Everitt BJ, Theodorsson-Norheim E et al (1984) Occurrence of neurotensin-like immunoreactivity in subpopulations of hypothalamic, mesencephalic and medullary catecholamines. J Comp Neurol 222, 543-559

Huntington G (1872) On chorea. Med Surg Report XXVI, 320-321

Inada J, Okuno E, Kimura M et al (1984) Intracellular localisation and characterisation of 3-hydroxykynurenine in human liver. Int J Biochem 16, 623-628

Itzhak Y, Stein, I (1990) Sigma binding sites in the brain; an emerging concept for multiple sites and their relevance for psychiatric disorders. Life Sci 47, 1073-1081

Iversen LL, Bird ED, Spokes EGS et al (1979) Agonist specificity of GABA binding sites in Huntington's disease and schizophrenia. In: GABA-Neurotransmitters (eds. Krogsgaard P, Scheel-Kruger J, Kofod H) Munhsgaard, Copenhagen

Iversen LL, Rossor MN, Reynolds GP (1983) Loss of pigmented dopamine-β-hydroxylase positive cells from locus coeruleus in senile dementia of Alzheimer's type. Neurosci Lett 39, 95-100

Jackson A, Crossman AR (1984) Experimental choreoathetosis produced by the injection of a gamma-aminobutyric acid antagonist into the lentiform nucleus in the monkey. Neurosci Lett 46, 41-45

Jacobs WA (1987) O-phthalaldehyde-sulfite derivatization of primary amines for liquid chromatography-electrochemistry. J Chromatogr 392, 435-441

Jakoby WB, Bonner DM (1951) A direct microbiological assay for quinolinic acid. J Biol Chem 191, 543-546

Jankovic J (1982) Treatment of hyperkinetic movement disorders with tetrabenazine: a double-blind crossover study. Ann Neurol 11, 41-47

Jeste DV, Barban L, Parisi J (1984) Reduced Purkinje cell density in Huntington's disease. Exp Neurol 85, 78-86

Jhamandas K, Boegman RJ, Beninger RJ et al (1990) Quinolinate-induced cortical cholinergic damage: modulation by tryptophan metabolites. Brain Res 529, 185-191

Jones EG, Hendry SHC (1986) Colocalisation of GABA and neuropeptides in neocortical neurons. Trends Neurosci 9, 71-76

Joseph MH, Baker HF, Crow TJ (1979) Brain tryptophan metabolism in schizophrenia: a post mortem study of metabolites on the serotonin and kynurenine pathways in schizophrenics and control subjects. Psychopharmacol 62, 279-285

Joyce JN, Lexow N, Bird ED et al (1988) Organisation of dopamine D1 and D2 receptors in human striatum: Receptor autoradiographic studies in Huntington's and schizophrenia . Synapse 2, 546-557

Kalen P, Strecker RE, Rosengren E et al (1989) Regulation of striatal serotonin release by the lateral habenula-dorsal raphe pathway in the rat as demonstrated by in vivo microdialysis: role of excitatory amino acids and GABA. Brain Res 492, 187-202

Kanazawa I, Kimura M, Murata M et al (1990) Choreic movements in the macaque monkey induced by kainic lesions of the striatum combined with L-Dopa. Pharmacological, biochemical and physiological studies on neural mechanisms. Brain 113, 509-535

Kawai J, Okuno E, Kido R (1988) Organ distribution of rat kynureninase and changes of its activity during development. Enzyme 39, 181-189

Kemp JA, Foster AC, Wong EHF (1987) Non-competitive antagonists of excitatory amino acid receptors. Trends Neurosci 10, 294-299

Kim JP, Choi DW (1987) Quinolinate neurotoxicity in cortical cell culture. Neurosci 23, 423-432

Kish SL, Shannak KS, Perry TL et al (1983) Neuronal (3H) benzodiazepine binding and levels of GABA, glutamate, and taurine are normal in Huntington's disease cerebellum. J Neurochem 41, 1495-1497

Kish SJ, Shannak K, Hornykiewicz O (1987) Elevated serotonin and reduced dopamine in subregionally divided Huntington's disease striatum. Ann Neurol 22, 386-389

Kish SJ, Du F, Parks DA et al (1991) Quinolinic acid is increased in cerebellum of patients with dominantly inherited olivipontocerebellar atrophy. Ann Neurol 29, 100-104

Klawans HL, Rubovits R (1972) Central cholinergic-anticholinergic antagonism in Huntington's chorea. Neurol 22, 107-116

Kleckner NW, Dingledine R (1988) Requirement for glycine in activation of NMDA-receptors in xenopus oocytes. Science 241, 835-837

Koh J-Y, Peters S, Choi DW (1986) Neurons containing NADPH-diaphorase are selectively resistant to quinolinate toxicity. Science 234, 73-76

Kohler C, Schwarcz R (1981) Monosodium glutamate: Increased neurotoxicity after removal of neuronal uptake sites. Brain Res 211, 485-491

Kohler C, Eriksson LG, Okuno E et al (1988a) Localisation of quinolinic acid metabolising enzymes in the rat brain. Immunohistochemical studies using antibodies to 3-hydroxyanthranilic acid oxygenase and quinolinic acid phosphoribosyltransferase. Neurosci 27, 49-76

Kohler C, Peterson A, Eriksson LG et al (1988b) Immunohistochemical identification of quinolinic acid phosphoribosyltransferase in glial cultures from the rat brain. Neurosci Lett 84, 115-119

Korbitz BC, Price JM, Brown RR et al (1963) Quantitative studies on tryptophan metabolism in the pyridoxine-deficient rat. J Nutr 80, 55-59

Kornhuber J, Kornhuber ME (1986) Presynaptic dopaminergic modulation of cortical input to the striatum. Life Sci 39, 669-674

Kornhuber J, Wichart I, Riederer P et al (1988) Kynurenine in hepatic encephalopathy. In: Quinolinic Acid and the Kynurenines 275-281 (ed: Stone TW) CRC Press, Boca Raton, Florida

Kowall NW, Ferrante RJ, Martin JB (1987) Patterns of cell losses in Huntington's disease. Trends Neurosci 10, 24-29

Kremer HPH, Roos RAC, Frolich M et al (1989) Endocrine functions in Huntington's disease. A two-and-a-half years follow-up study. J Neurol Sci 90, 335-344

Kremer HPH, Roos RAC, Dingjan G et al (1990) Atrophy of the hypothalamic lateral tuberal nucleus in Huntington's disease. J Neuropath Exp Neurol 49, 371-382

Kremzner LT, Berl S, Stellar S et al (1979) Amino acids, peptides, and polyamines in cortical biopsies and ventricular fluid in patients with Huntington's disease. Adv Neurol 23, 537-546

Kuhl DE, Metter EJ, Riege WH et al (1984) Patterns of cerebral glucose utilisation in Parkinson's disease and Huntington's disease. Ann Neurol 15 Suppl S119-S125

Kurlan R, Caine E, Rubin A et al (1988) Cerebrospinal fluid correlates of depression in Huntington's disease. Arch Neurol 45, 881-883.

Kuwert T, Lange HW, Langen K-J et al (1989) Cerebral glucose consumption measured by PET in patients with and without psychiatric symptoms of Huntington's disease. Psychiatr Res 29, 361-362

Kuwert T, Lange HW, Langen K-J et al (1990) Cortical and subcortical glucose consumption measured by PET in patients with Huntington's disease. Brain 113, 1405-1423

Lane JD, Smith JE, Shea PA (1976) Neurochemical changes following the administration of depleters of biogenic monoamines. Life Sci 19, 1663-1667

Lange HW, Thorner G, Hopf A et al (1976) Morphometric studies of the neuropathological changes in choreatic diseases. J Neurol Sci 28, 401-425

Lange HW (1981) Quantitative changes of telencephalon, diencephalon, and mesencephalon in Huntington's chorea, postencephalitic, and idiopathic Parkinsonism. Verh Anat Ges 75, 923-925

Lapin IP (1981) Kynurenines and seizures. Epilepsia 22, 257-265

Lapin IP, Rysov IV (1989) Receptors involved in excitatory effects of kynurenines. Pharmacol i Toxicol (Moscow) 51/1, 98-104

Lapin IP, Rysov IV (1990) Effect of catecholaminergic drugs on quinolinate- and kynurenine-induced seizures in mice. J Neural Trans 82, 55-65

Lebrun-Grandie P, Kien P, Tison F et al (1988) MRI evaluation of movement disorders. Abstract: P26 Neural Mechanisms in Disorders of Movement

Lee DK, Markham CH, Clark WG (1968) Serotonin (5HT) metabolism in Huntington's chorea. Life Sci 7, 707-712

Lees GJ (1987) Effects of ketamine on the in vivo toxicity of quinolinate and N-methyl-D-aspartate in the rat hippocampus. Neurosci Lett 78, 180-186

Legidio A, Clancy RR, Berman PH (1988) Recent

advances in the diagnosis, treatment and prognosis of neonatal seizures. Ped Neurol 4, 79-86

Lehman J, Schaefer P, Ferkany JW et al (1983) Quinolinic acid evoked (3H) acetylcholine release in striatal slices: Mediation by NMDA-type excitatory amino acid receptors. Eur J Pharmac 96, 111-115

Levey AI, Wainer BH, Mufson EJ et al. (1983) co-localization of acetylcholinesterase and choline acetyltransferase in rat cerebrum. Neurosci 9, 9-22

Lloyd KG, Dreksler S, Bird ED (1977) Alterations in (3H) GABA in Huntington's chorea. Life Sci 21, 747-754

Lloyd KG, Munari C, Bossi L et al (1980) Function and dysfunction of the GABA system in the human brain. In: Transmitter Biochemistry of Human Brain Tissue 127-141 (eds. Riederer P, Usdin, E) McMillan Publishers Ltd, London.

Lloyd KG, Zivkovic B, Scatton B et al (1989) The GABAergic hypothesis of depression. Prog Neuro-Psychopharmacol Biol Psychiatr 13, 341-351

Lombardi G, Zanoni R, Moroni F (1989) Systemic treatments with GM1 gangliosides reduce quinolinic acid-induced striatal lesions in the rat. E J Pharmacol 174, 123-125

McCaughey WTE (1961) The pathological spectrum of Huntington's chorea. J Nerv Ment Dis 133, 91-103

McGeer EG, Singh E (1984) Neurotoxic effects of endogenous materials: Quinolinic acid, L-pyroglutamate and TRH. Expl Neurol 86, 410-413

McGeer EG, McGeer PL (1976a) Duplication of biochemical changes of Huntington's chorea by intrastriatal injections of glutamic and kainic acids. Nature 263, 517-518

McGeer PL, McGeer EG (1976b) Enzymes associated with the metabolism of catecholamines, acetylcholine and GABA in human controls and patients with Parkinson's disease and Huntington's chorea. J Neurochem 26, 65-76

McHugh PR and Folstein MF (1975) Psychiatric syndromes of Huntington's chorea: A clinical and phenomenologic study. In: Psychiatric aspects of neurologic diseases 267-285 (ed: Benson DF, Blumer D) Grune and Stratton Inc, New York

McLean DR (1982) Failure of isoniazid therapy in Huntington's disease. Neurol 32, 1189-1191

McLeod WR, Horne DJ de L (1972) Huntington's chorea and tryptophan. J Neurol Neurosurg Psychiat 35, 510-513

Magnussen I, Dupont E, Prange-Hansen A et al (1977) Palatal myoclonus treated with 5-hydroxytryptophan and a decarboxylase inhibitor. Acta Neurol Scand 55, 251-253

Majewska MD, ffrench-Mullen JMH, London ED (1989) Ascorbic acid and glutathione are antagonists of the NMDA receptor. Soc Neurosci Abstract 463.15

Manango RM, Schwarcz R (1983) Chronic infusion of endogenous excitatory amino acids into rat striatum and hippocampus. Brain Res Bull 10, 47-51

Mann DMA (1989) Subcortical afferent projection systems in Huntington's chorea. Acta Neuropath 78, 551-554

Mann JJ, Kaplan RD, Bird ED (1986) Elevated postmortem monamine oxidase B activity in the caudate nucleus in Huntington's disease compared to schizophrenics and controls. J Neural Trans 65, 277-283

Mann VM, Cooper JM, Javoy-Agid Y et al (1990) Mitochondrial function and parental sex effect in Huntington's disease. Lancet 336, 749

Manthey MK, Pyne SG, RJW Truscott (1990) Mechanism of reaction of 3-hydroxyanthranilic acid with molecular oxygen. Biochimica Biophysica Acta 1034, 207-212

Martin JB (1984) Huntington's disease: new approaches to an old problem. Neurology 34, 1059-1072

Martin JR, Dedek J, Driscoll P (1983) Portocaval anastomosis in rats: effects on behaviour and brain serotonin metabolism. Pharmacol Biochem Behav 18, 269-272

Mattson B, Persson SA (1973) Cerebrospinal 5-HIAA levels in Huntington's chorea. Adv Neurol 1, 557-558

Mayer ML, Vylicky L, Clements J (1989) Regulation of NMDA receptor desensitization in mouse hippocampal neurons by glycine. Nature 338, 425-427

Mazzari S, Aldinio C, Beccaro M et al (1986) Intracerebral quinolinic acid injection in the rat: effects on dopaminergic neurons. Brain Res 380, 309-316

Mazziotta JC, Phelps ME, Pahl JJ et al (1987) Reduced cerebral glucose metabolism in asymptomatic subjects at risk for Huntington's disease. N Eng J Med 316, 357-362

Melamed E, Hefti F, Bird ED (1982) Huntington's chorea is not associated with hyperactivity of nigrostriatal dopaminergic neurons: studies in postmortem tissues and in rats with kainic acid lesions. Neurol 32, 640-644

Mitchell IJ, Jackson A, Sambrook MA et al (1989) The role of the subthalamic nucleus in experimental chorea. Brain 112, 1533-1548

Monaghan DT, Cotman CW (1985) Distribution of N-methyl-D-aspartate-sensitive L-(3H) glutamate binding sites in rat brain. J Neurosci 5, 2909-2919

Morales LM, Estevez J, Suarez H et al (1989) Nutritional evaluation of Huntington's disease patients. Am J Clin Nutr 50, 145-50

Moroni F, Lombardi G, Carla V et al (1984a) The excitotoxin quinolinic acid is present and unevenly distributed in the rat brain. Brain Res 295, 352-355

Moroni F, Lombardi G, Moneti G et al (1984b) The excitotoxin quinolinic acid is present in the brain of several mammals and its cortical content increases during the aging process. Neurosci Lett 47, 51-55

Moroni F, Lombardi G, Carla, V (1985) Changes of the brain content of quinolinic acid: possible correlation with brain pathology. Soc Neurosci Abstract 821, 241.1

Moroni F, Lombardi G, Carla V et al (1986a) Increase in the content of quinolinic acid in cerebrospinal fluid and frontal cortex of patients with hepatic failure. J Neurochem 47, 1667-1671

Moroni F, Lombardi G, Carla V et al (1986b) Content of quinolinic acid and of other tryptophan metabolites increases in brain regions of rats used as experimental models of hepatic encephalopathy. J Neurochem 46, 869-874

Moroni F, Lombardi G, Robitaille Y et al (1986c) Senile dementia and Alzheimer's disease: lack of changes of the cortical content of quinolinic acid. Neurobiol Aging 7, 249-253

Moroni F, Carla V, Russi P (1988a) Changes of kynurenic acid content in the rat brain during the development and aging processes, at different hours

of the day and after the admnistration of precursors. Psychopharmacol Suppl 96, FR 01.01

Moroni F, Russi P, Lombardi G et al (1988b) Presence of kynurenic acid in the mammalian brain. J Neurochem 51, 177-180

Mostafa MH, El-Sewedy SM, El-Bassiouni EA et al (1982) In vivo and in vitro studies on the effects of some phenothiazines and sulpiride on kynurenine metabolism. Biochem Pharmacol 31, 2227-2230

Mountjoy CQ, Rossor MN, Iversen LL et al (1984) Correlation of cortical cholinergic and GABA deficits with quantitative neuropathological findings in senile dementia. Brain 107, 507-518

Nadler JV (1981) Mini review: Kainic acid as a tool for the study of temporal lobe epilepsy. Life Sci 29, 2031-2042

Naito J, Ishiguro I, Murazumi T et al (1987) Determination of kynurenine in serum by high-performance liquid chromatography after enzymatic conversion to 3-hydroxykynurenine. Anal Biochem 161, 16-19

Naoi M, Ishiki R, Nomura Y et al (1987) Quinolinic acid: an endogenous inhibitor specific for type B monoamine oxidase in human brain synaptosomes. Neurosci Lett 74, 232-236

Nasu S, Yamaguchi K, Sakakibara S et al (1981) The effect of pyrazines on the metabolism of tryptophan and nicotinamide adenine dinucleotide in the rat. Biochim Biophysica Acta 677, 109-119

Nemeroff CB, Youngblood WW, Manberg PJ et al (1983) Regional brain concentrations of neuropeptides in Huntington's chorea and schizophrenia. Science 331, 972-975

Nutt JG, Gillespie MM, Chase TN (1979) Treatment of Huntington's disease with alpha- and beta-adrenergic antagonists. Adv Neurol 23, 777-784

Ogihara K, Mozai T, Hirai SN (1966) Tryptophan as a cause of hepatic coma. New Engl J Med 275, 1255

Oka H (1980) Organisation of the cortico-caudate projections. Exp Brain Res 40, 203-208

Okuno E, Schwarcz R (1985) Purification of quinolinic acid phosphoribosyltransferase from rat liver and brain. Biochim Biophysic Acta 841, 112-119

Okuno E, Kohler C, Schwarcz R (1987) Rat 3-hydroxyanthranilic acid oxygenase: purification from the liver and immunocytochemical localisation in the brain. J Neurochem 49, 771-780

Okuno E, Ishikawa T, Tsujimoto M et al (1990) Purification and characterisation of kynurenine-pyruvate aminotransferase from rat kidney and brain. Brain Res 534, 37-44

Okuno E, Nakamura M, Schwarcz R (1991) Two kynurenine aminotransferases in human brain. Brain Res 542, 307-312

Oldroyd J, Wagster UV, Folstein S et al (1990) Dopamine uptake sites are increased in Huntington's disease neostriatum. Neurol 40, Suppl 808P

Olney JW (1971) Glutamate-induced neuronal necrosis in the infant mouse hypothalamus. J Neuropath Exp Neurol 30, 75-90

Olsen RW, Van Ness PC, Tourtelotte WW (1979) Gamma-aminobutyric acid receptor binding curves of human brain regions: comparison for Huntington's disease and normal. Adv Neurol 23, 697-704

Olsen RW (1981) GABA-benzodiazepine-barbiturate receptor interactions. J Neurochem 37, 1-13

Oyanagi K, Takeda S, Takahashi H et al (1989) A quantitative investigation of the substantia nigra in Huntington's disease. Ann Neurol 26, 13-19

Palmer AM, Stratmann GC, Procter AW et al (1988) Possible neurotransmitter basis of behavioral changes in Alzheimer's disease. Ann Neurol 23, 616-620

Parker WD, Boyson SJ, Luder AS et al (1990) Evidence for a defect in NADH: ubiquinone oxidoreductase (complex I) in Huntington's disease. Neurol 40, 1231-1234

Patterson JI, Brown RR (1980) Determination of quinolinic acid by high-performance liquid chromatography. J Chromatogr 182, 425-429

Pearson SJ, Reynolds GP (1988) Depletion of monoamine transmitters by tetrabenazine in brain tissue in Huntington's disease. Neuropharmacol 27, 711-719

Pearson SJ, Reynolds GP (1989) Tryptophan metabolism and quinolinic acid in the brain in Huntington's disease. In: Neural Mechanisms in Disorders of Movement 343-346 (eds. Crossman AR, Sambrook MA)

Libbey, London

Pearson SJ, Heathfield KWG, Reynolds GP (1990) Pallidal GABA and chorea in Huntington's disease. J Neural Trans 81, 241-246

Pearson SJ, Reynolds GP (1991) The determination of 3-hydroxykynurenine in human brain and plasma by high-performance liquid chromatography with electrochemical detection. Increased concentrations in hepatic encephalopathy. J Chromatogr (Biomed Appl) 565, 436-440

Pearson SJ, Czudek C, Mercer K et al (1991) Electrochemical detection of human brain transmitter amino acids by high-performance liquid chromatography of stable o-phthalaldehyde-sulphite derivatives. J Neural Trans 86, 151-157

Peiris JB, Boralessa H, Lionel NDW (1976) Clonazepam in the treatment of choreiform activity. Med J Aus 1, 225-227

Penney JB, Young AB (1982) Quantitative autoradiography of neurotransmitter receptors in Huntingon's disease. Neurol 32, 1391-1395

Penney JB, Young AB (1983) Speculations on the functional anatomy of basal ganglia disorders. Ann Rev Neurosci 6, 73-94

Perkins MN and Stone TW (1983a) Pharmacology and regional variations of quinolinic acid-evoked excitations in the rat central nervous system. J Pharmacol Exp Therap 226, 551-557

Perkins MN and Stone TW (1983b) On the interaction of 2-amino-7-phosphono-heptanoic acid and quinolinic acid in mice. Eur J Pharmac 89, 297-300

Perkins MN and Stone TW (1983c) Quinolinic acid: Regional variations in neuronal sensitivity. Brain Res 247, 184-187

Perry EK, Perry RH, Tomlinson BE (1982) The influence of agonal status on some neurochemical activities of postmortem human brain tissue. Neurosci Lett 29, 303-307

Perry RH, Tomlinson BE, Candy JM et al (1983) Cortical cholinergic and GABA deficits in mentally impaired Parkinsonian patients. Lancet ii, 789-790

Perry TL, Hansen S, Kloster M (1973) Deficiency of γ-aminobutyric acid in brain. N Eng J Med 288, 337-342

Perry TL, Bratty PJ, Hansen S (1975) Hereditary

mental depression and Parkinsonism with taurine in the central nervous system. Arch Neurol 32, 108-113

Perry TL, Wright JM, Hansen S et al (1980) Failure of aminooxyacetic acid therapy in Huntington's disease. Neurol 30, 772-775

Perry TL, Hansen S, Gandham SS (1981) Postmortem changes of amino compounds in human and rat brain. J Neurochem 36, 406-412

Perry TL (1982) Normal cerebrospinal fluid and brain glutamate levels in schizophrenia do not support the hypothesis of glutamatergic neuronal dysfunction. Neurosci Lett 28, 81-85

Perry TL, Yong VW, Hansen S et al (1987) Tissue culture evidence for a circulating neurotoxin in Huntington's chorea. J Neurol Sci 78, 139-150

Perry TL, Hansen S (1990) What excitotoxin kills striatal neurons in Huntington's disease? Clues from neurochemical studies. Neurol 40, 20-24

Peters S, Koh J, Choi DW (1987) Zinc selectively blocks the action of N-methyl-D-aspartate on cortical neurones. Science 236, 589-593

Pettegrew JW, Nichols JS, Stewart RM (1987) Membrane studies in Huntington's disease: steady state and time-dependent fluorescence spectroscopy of intact lymphocytes. J Neurochem 36, 1966-1976

Pettibone DJ, Totaro JA, Pflueger AB (1984) Tetrabenazine-induced depletion of brain monoamines: characterisation and interaction with selected antidepressants. Eur J Pharmacol 102, 425-430

Peyser CE, Folstein SE (1990) Huntington's disease as a model for mood disorders. Clues from neuropathology and neurochemistry. Molec Chem Neuropath 12, 99-119

Peyser CE (1991) Abstract: World Fed Neurol Research Group on Huntington's chorea

Pflanz S, Besson JAO, Ebmeier KP et al (1991) The clinical manifestation of mental disorder in Huntington's disease: a retrospective case record of disease. Acta Psychiatr Scand 83, 53-60

Phillipson OT, Bird ED (1976) Plasma growth hormone concentrations in Huntington's chorea. Clin Sci Molec Med 50, 551-554

Phillipson OT, Bird ED (1977) Plasma glucose, non-esterified fatty acids and amino acids in Huntington's chorea. Clin Sci Molec Med 52, 311-318

Podolsky S, Leopold NA, Sax DS (1972) Increased frequency of diabetes mellitus in patients with Huntington's chorea. Lancet i, 1356-1358

van Praag HM (1977) Depression and Schizophrenia, Spectrum Publications, New York

van Praag HM, Plutchik R, Conte H (1986) The serotonin hypothesis of (auto)aggression. Ann N Y Acad Sci 487, 150-167

Procter AW, STirling JM, Stratmann GC et al (1989) Loss of glycine-dependent radioligand binding to the N-methyl-D-aspartate-phencyclidine receptor complex in patients with Alzheimer's disease. Neurosci Lett 101, 62-66

Quagliarello E, Papa S, Saccone C et al (1964) Effect of 3-hydroxyanthranilic acid on the mitochondrial respiratory system. Biochem J 91, 137-146

Ransom RW, Stec NL (1988) Co-operative modulation of (3H) MK8OI binding to the N-methyl-D-aspartate receptor-ion channel complex by L-glutamate, glycine and polyamines. J Neurochem 51, 830-836

Rapoport MI, Beisel WR, Hornick RB (1970) Studies of tryptophan metabolism in experimental animals and man during infectious illness. Am J Clin Nutr 24, 807-814

Reches A, Burke RE, Kuhn CM et al (1983) Tetrabenazine, an amine depleting drug also blocks dopamine receptors in rat brain. J Pharmacol Exp Ther 225, 515-521

Reddi OS, Reddy VR, Reddy KRS (1978) Familial hydroxykynureninuria. Hum Hered 28, 238-240

Reibling A, Reyes P, Jameson KD (1975) Dimethylaminoethanol ineffective in Huntington's disease. New Eng J Med 293, 724

Reid IC, Besson JAO, Best PV et al (1988) Imaging of cerebral blood flow markers in Huntington's disease using single photon emission computed tomography. J Neurol Neurosurg Psychiat 51, 1264-1268

Reiner A, Albin Rl, Anderson KD et al (1988) Differential loss of striatal projection neurons in Huntington's disease. Proc Natl Acad Sci 85, 5733-5737

Reisberg B, Feeris SH, de Leon MJ et al (1982) Global deterioration scale for age-associated cognitive decline and Alzheimer's disease. Am J

Psychiat 139, 1136-1139

Reisine TD, Fields JZ, Stern LZ et al (1977) Alterations in dopaminergic receptors in Huntington's disease. Life Sci 21, 1123-1128

Reisine TD, Wastek GJ, Speth RC et al (1978) Alterations in the benzodiazepine receptor of Huntington's diseased human brain. Brain Res 165, 183-187

Reisine TD, Beaumont K, Bird ED et al (1979) Huntington's disease: Alterations in neurotransmitter receptor binding in the human brain. Adv Neurol 23

Reisine TD, Overstreet D, Gale K et al (1980) Benzodiazepine receptors: the effect of GABA on their characteristics in human brain and their alteration in Huntington's disease. Brain Res 199, 79-88

Reynolds GP (1983) Increased concentrations and lateral asymmetry of amygdala dopamine in schizophrenia. Nature 305, 527-529

Reynolds GP, Garrett NJ (1986) Striatal dopamine and homovanillic acid in Huntington's disease. J Neural Trans 65, 151-155

Reynolds GP, Pearson SJ (1987a) Decreased glutamate and increased serotonin in Huntington's disease. Neurosci Lett 78, 233-238

Reynolds GP, Pearson SJ (1987b) Neuropathology of Huntington's disease. Trends Neurosci 10, 404

Reynolds GP, Pearson SJ, Halket J, Sandler M (1988) Brain quinolinic acid in Huntington's disease. J Neurochem 50, 1959-1960

Reynolds GP (1989) Beyond the dopamine hypothesis: The neurochemical pathology of schizophrenia. B J Psychiatr 155, 305-316

Reynolds GP, Pearson SJ (1989) Increased brain 3-hydroxykynurenine in Huntington's disease. Lancet ii, 979-980

Reynolds GP, Pearson SJ, Heathfield KWG (1990a) Dementia in Huntington's disease is associated with neurochemical deficits in the caudate nucleus, not the cerebral cortex. Neurosci Lett 113, 95-100

Reynolds GP, Brown JE, Pearson SJ (1990b) No deficit of pallidal D2 receptors in Huntington's disease. J Neurol Sci 100, 238

Reynolds GP, Czudek C, Andrews HB (1990c) Deficit and hemispheric asymmetry of GABA uptake sites in the hippocampus in schizophrenia. Biol Psychiatr 27, 1038-1044

Reynolds GP, Pearson SJ (1990) Brain GABA levels in asymptomatic Huntington's disease. New Eng J Med 323, 682

Reynolds GP, CEJ Warner (1988) Amino acid neurotransmitter deficits in adult Down's syndrome brain tissue. Neurosci Lett 94, 224-227

Richardson EP (1990) Huntington's disease: Some recent neuropathological studies. Neuropath Appl Neurobiol 16, 451-460

Rieke GK, Scarfe AD, Hunter JF (1984) L-pyroglutamate: An alternative neurotoxin for a rodent model of Huntington's disease. Brain Res Bull 13, 443-456

Rieke GK, Smith J, Idusuyi J et al (1989) Chronic intrastriatal L-pyroglutamate: Neuropathology and neurology sparing like Huntington's disease. Exp Neurol 104, 147-154

Ringel SP, Weiner WJ, Rubovits R (1973) Methysergide in Huntington's chorea. Adv Neurol 1, 769-775

Roberts PJ, Andersen SD (1979) Stimulatory effect of L-glutamate and related amino acids on (3H) dopamine release from rat striatum: An in vitro model for glutamate actions. J Neurochem 32, 1539-1545

Robertson RG, Farmery SM, Sambrook MA et al (1989) Dyskinesia in the primate following injection of an excitatory amino acid antagonist into the medial segment of the medial pallidum. Brain Res 476 317-322

Robinson MB, Anderson KD, Koerner JF (1984) Kynurenic acid as an antagonist of hippocampal excitatory transmission. Brain Res 309, 119-126

Rodda RA (1981) Cerebellar atrophy in Huntington's disease. J Neurol Sci 50, 147-157

Roisin L, Stellar S, Liu JC (1979) Neuronal nuclear-cytoplasmisc inclusions in Huntington's chorea: electron microscope investigations. Adv Neurol 23, 95-122

Roos RAC (1991) Abstract: World Fed Neurol Research Group on Huntington's chorea

Rossi-Fanelli F, Freund H, Krause R et al (1982) Induction of coma in normal dogs by the infusion of

aromatic amino acids and its prevention by the addition of branched-chain amino acids. Gastroenterology 83, 664-671

Rossor MN, Iversen LL, Reynolds GP et al (1984) Neurochemical characteristics of early and late onset types of Alzheimer's disease. B Med J 288, 961-964

Rothman SM, Olney JW (1987) Excitotoxicity and the NMDA receptor. Trends Neurosci 10, 299-302

Rothstein JD, McKhann, Guarneri P et al (1989) Cerebrospinal fluid content of diazepam binding inhibitor in chronic hepatic encephalopathy. Ann Neurol 26, 57-62

Saito K, Markey SP, Heyes MP (1991) Chronic effects of γ-interferon on quinolinic acid and indoleamine-2,3-dioxygenase in brain of C57BL6 mice. Brain Res 546, 151-154

Sajatovic M, Verbanac P, Ramirez LF et al (1991) Clozapine treatment of psychiatric symptoms resistant to neuroleptic treatment in patients with Huntington's chorea. Neurol 41, 156

Sanberg PR, Fibiger HC (1979) Body weight, feeding, and drinking behaviours in rats with kainic acid-induced lesions of striatal neurons- with a note on body weight symptomology in Huntington's disease. Exp Neurol 66,444-466

Sanberg PR, Johnston GA (1981) Glutamate and Huntington's disease. Med J Aust 2, 460-465

Sanberg PR, Calderon SF, Garver DL et al (1987) Brain tissue transplants in an animal model of Huntington's disease. Psychopharmacol Bull 23, 476-482

Sanberg PR, Calderon SF, Giordano M et al (1989) The quinolinic acid model of Huntington's disease: locomotor abnormalities. Exp Neurol 105, 45-53

Sasaki H, Muramoto O, Kanazawa I et al (1986) Regional distribution of amino acid transmitters in postmortem brains of presenile and senile dementia of Alzheimer type. Ann Neurol 19, 263-269

Schmitt ML, Graeff FG, Carobrez AP (1990) Anxiolytic effect of kynurenic acid microinjected into the dorsal periaqueductal gray matter of rats placed in the elevated plus-maze test. Braz J Med Biol Res 23, 677-679

Schwarcz R, Kohler C (1983) Differential vulnerability of central neurons of the rat to

quinolinic acid. Neurosci Lett 38, 85-90

Schwarcz R, Whetsell WO, Mangano RM (1983) Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. Science 219, 316-318

Schwarcz R, Brush GS, Foster AC et al (1984a) Seizure activity and lesions after intrahippocampal quinolinic acid injection. Expl Neurol 84, 1-17

Schwarcz R, Foster AC, French ED et al (1984b) Excitotoxic models for neurodegenerative disorders. Life Sci 35, 19-32

Schwarcz R, Meldrum B (1985) Excitatory amino acid antagonists provide a therapeutic approach to neurological disorders. Lancet ii, 140-143

Schwarcz R, Okuno E, White RJ et al (1988a) 3-Hydroxyanthranilate oxygenase activity is increased in the brains of Huntington's disease victims. Proc Natl Acad Sci 85, 4079-4081

Schwarcz R, Tammings CA, Kurlan R (1988b) Cerebrospinal fluid levels of quinolinic acid in Huntington's disease and schizophrenia. Ann Neurol 24, 580-582

Schwarcz R, Okuno E, White RJ (1989) Basal ganglia lesions in the rat: effects on quinolinic acid metabolism. Brain Res 490, 103-109

Seeman P, Bzowej NH, Guan HC et al (1987) Human brain D1 and D2 dopamine receptors in schizophrenia, Alzheimer's, Parkinson's, and Huntington's diseases. Neuropsychopharmacol 1, 5-15

Seeman P, Niznik HB, Guan H-C et al (1989) Link between D1 and D2 dopamine receptors is reduced in schizophrenia and Huntington's diseased brain. Proc Natl Acad Sci 86, 10156-10160

Seizinger BR, Leibisch DC, Kish SJ et al (1986) Opioid peptides in Huntington's disease: Alterations in prodynorphin and proenkephalin system. Brain Res 378, 405-408

Seto-Ohshima A, Emson PC, Carassco LH et al (1988a) Selective damage to the striatal matrix in Huntington's disease. Abstract P29: Neural Mechansims in Disorders of Movement

Seto-Ohshima A, Emson PC, Lawson E et al (1988b) Loss of the matrix calcium-binding protein-containing neurons in Huntington's disease. Lancet ii, 1252-1255

Shannak KS, Hornykiewicz O (1980) Brain monoamines in the rhesus monkey during long-term neuroleptic adminstration. Adv Biochem Psychopharmacol 24, 315-323

Shapira AHV, Cooper JM, Dexter D et al (1989) Mitochondrial complex I deficiency in Parkinson's disease. Lancet i, 1296

Shoulson I (1978) Huntington's disease: Treatment with muscimol, a GABA-mimetic drug. Ann Neurol 4, 279-284

Shoulson I, Odoroff C, Oakes D et al (1989) A controlled clinical trial of baclofen as protective therapy in early Huntington's disease. Ann Neurol 25, 252-259

Shults C, Steardo L, Barone P et al (1986) Huntington's disease: Effect of cysteamine, a somatostatin-depleting agent. Neurol 36, 1099-1102

Simmons JT, Pastakia B, Chase TN et al (1988) Magnetic resonance imaging in Huntington's disease. Am J Neuroradiol 7, 25-28

Simon RP, Swan JH, Griffiths T et al (1984) Blockade of N-methyl-D-aspartate receptors may protect against ischaemic damage in the brain. Science 826, 850-856

Simon RP, Schmidley JW, Meldrum BS et al (1986) Excitotoxic mechanisms in hypoglycaemic hippocampal injury. Neuropath Appl Neurobiol 12, 567-576

Simpson MDC, Cross AJ, Slater P et al (1987) (3H) Nipecotic acid binding to GABA uptake sites in Huntington's disease. B J Pharmacol 93, P158

Sotrel A, Myers RH (1990) Morphometric analysis of prefrontal cortex in Huntington's disease. J Neuropath Exp Neurol 49, 346

Soubrie P, Reisine TD, Glowinski J (1984) Functional aspects of serotonin transmission in the basal ganglia: A review and an in vivo approach using the push-pull cannula technique. Neuroscience 13, 605-625

Sourkes TL, Missala K, Oravec M (1970) Decrease of cerebral serotonin and 5-hydroxyindolyacetic acid caused by (-)-α-methyltryptophan. J Neurochem 17, 111-115

Sourkes TL (1978) Tryptophan in hepatic coma. J Neural Trans Suppl 14, 79-86

Speciale C, Okuno E, Schwarcz R (1987) Increased

quinolinic acid metabolism following neuronal degeneration in the rat hippocampus. Brain Res 436, 18-24

Speciale C, Schwarcz R (1988) Effect of systemic kainate administration on cerebral quinolinic acid metabolism in the rat. Exp Neurol 99, 213-218

Speciale C, Hares K, Schwarcz R et al (1989a) High-affinity uptake of L-kynurenine by a Na+ - independent transporter of neutral amino acids in astrocytes. J Neurosci 9, 2066-2072

Speciale C, Ungerstedt U, Schwarcz R (1989b) Production of extracellular quinolinic acid in the striatum studied by microdialysis in unanesthetised rats. Neurosci Lett 104, 345-350

Speciale C, Schwarcz R (1990) Uptake of kynurenine into rat brain slices. J Neurochem 54, 156-163

Speciale C, Wu H-Q, Gramsbergen JBP et al (1990) Determination of extracellular kynurenic acid in the striatum of unanesthatised rats: effect of aminooxyacetic acid. Neurosci Lett 116, 198-203

Sperk G, Reynolds GP, Riederer P (1987) HPLC analysis of somatostatin related peptides in putamen of Huntington's disease patients. J Neural Trans 69, 153-160

Spindel ER, Wurtman RJ, Bird ED (1980) Increased TRH content of the basal ganglia in Huntington's disease. New Eng J Med 303, 1235-1236

Spokes EGS (1979) An analysis of factors influencing measurements of dopamine, noradrenaline, glutamate decarboxylase and choline acetylase in human post mortem brain. Brain 102, 333-346

Spokes EGS, Garrett NJ, Iversen LL (1979) Differential effects of agonal status on measurement of GABA and glutamate decarboxylase in human post-mortem brain tissue from control and Huntington's chorea subjects. J Neurochem 33, 773-778

Spokes EGS (1980) Neurochemical alterations in Huntington's chorea: a study of post-mortem brain tissue. Brain 103, 179-210

Spokes EGS, Garrett NJ, Rossor MN et al (1980) Distribution of GABA in post-mortem brain tissue from control, psychotic and Huntington's chorea subjects. J Neurol Sci 48, 303-313

Spokes EGS (1981) The neurochemistry of Huntington's chorea. Trends Neurosci 4, 115-118

Stahl WL, Swanson PD (1974) Biochemical abnormalities in Huntington's chorea brains. Neurol 24, 813-819

Starkstein SE, Brandt J, Folstein S et al (1988) Neuropyschological and neurological correlates in Huntington's disease. J Neurol Neurosurg Psychiat 51, 1259-1263

Starkstein SE, Folstein SE, Brandt J et al (1989) Brain atrophy in Huntington's disease. A CT-scan study. Neuroradiol 31, 156-159

Stevens CD, Altshuler LL, Bogerts B et al (1988) Quantitative study of gliosis in schizophrenia and Huntington's disease. Biol Psychiatr 24, 697-700

Stone TW and Perkins MN (1981) Quinolinic acid: A potent endogenous excitant at amino acid receptors in rat CNS. Eur J Pharmacol 72, 411-412

Stone TW, Connick JH (1985) Quinolinic acid and other kynurenines in the central nervous system. Neurosci 15, 597-617

Susel Z, Engber TM, Kuo S et al (1991) Prolonged infusion of quinolinic acid into rat striatum as an excitoxic model of neurodegenerative disease. Neurosci Lett 121, 234-238

Swartz KJ, During MJ, Freese A et al (1990a) Cerebral synthesis and release of kynurenic acid: an endogenous antagonist of excitatory amino acid receptors. J Neurosci 10, 2965-2973

Swartz KJ, Matson WR, MacGarvey UM et al (1990b) Measurement of kynurenic acid in mammalian brain extracts and cerebrospinal fluid by high-performance liquid chromatography with fluorometric and coulometric electrode array detection. Anal Biochem 185, 363-376

Takeuchi F, Otsuka H, Shibata Y (1980) Purification and properties of kynureninase from rat liver. J Biochem 88, 987-994

Tarsy D, Leopold N, Sax DS (1974) Physostygmine in choreiform movement disorders. Neurol 24, 28-33

Tellez-Nagel I, Johnson AB, Terry RD (1973) Ultrastructural and histochemical study of cerebral biopsies in Huntington's chorea. Adv Neurol 1, 387-398

Teulings FAG, Fokkens W, Kaalen JGAH et al (1973) The concentration of free and conjugated 3-hydroxyanthranilic acid in the urine of bladder tumour patients before and after therapy, measured

with an enzymatic method. B J Cancer 27, 316-322

Tomoda A, Shiraswa E, Yoneyama Y (1986) Reactions of oxy- and methemoglobin with tryptophan metabolites, 3-hydroxyanthranilic acid and 3-hydroxykynurenine. Hemoglobin 10, 33-44

Tork I, Hornung J-P, Somogyi P (1988) Serotonergic innervation of GABAergic neurons in the cerebral cortex. Neurosci Lett Suppl 30, S131

Toru M, Watanabe S, Shibuya H et al (1988) Neurotransmitters, receptors and neuropeptides in post-mortem brains of chronic schizophrenic patients. Acta Psychiatr Scand 78, 121-137

Toseland PA (1969) The determination of urinary quinolinic acid by gas liquid chromatography. Clin Chim Acta 25, 185-186

Trifeletti RR, Snowman AM, Whitehouse PJ et al (1987) Huntington's disease: increased number and altered regulation of benzodiazepine receptor complexes in frontal cerebral cortex. Neurol 37, 916-922

Turski WA, Nakamura M, Todd WP et al (1988) Identification and quantification of kynurenic acid in human brain tissue. Brain Res 454, 164-169

Turski WA, Gramsbergen JBP, Traitler H et al (1989) Rat brain slices produce and liberate kynurenic acid upon exposure to L-kynurenine. J Neurochem 52, 1629-1636

Uhlhaas S, Lange H (1988) Striatal deficiency of L-pyroglutamatic acid in Huntington's disease is accompanied by increased plasma levels. Brain Res 457, 196-199

Urquhart N, Perry TL, Hansen S et al (1975) GABA content and glutamic acid decarboxylase activity in brain of Huntington's chorea patients and control subjects. J Neurochem 24, 1071-1075

Vacca-Galloway LL, Nelson S (1984) Glial cells in Huntington's chorea. Adv Cell Neurobiol 5, 221-250

Vecsei L, Beal MF (1990) Intracerebroventricular injection of kynurenic acid, but not kynurenine induces ataxia and stereotyped behaviour in rats. Brain Res Bull 25, 623-627

Vestergaard P, Braastrup PC, Petersson H (1977) Lithium treatment of Huntington's chorea: A placebo-controlled clinical trial. Acta Psychiatr Scand 56, 183-188

Vezzani A, Wu HQ, Angelico P et al (1988) Quinolinic acid-induced seizures, but not nerve cell death, are associated with extracellular Ca 2+ decrease assessed in the hippocampus by brain dialysis. Brain Res 454, 289-297

Vezzani A, Serafini R, Stasi MA et al (1989) Kinetics of MK-801 and its effect on quinolinic acid-induced seizures and neurotoxicity. J Pharm Exp Therap 249, 278-283

Vezzani A, Gramsbergen JBP, Versari P et al (1990) Kynurenic acid synthesis by human glioma. J Neurol Sci 99, 51-57

Vezzani A, Forloni GL, Serafini R (1991) Neurodegenerative effects induced by chronic infusion of quinolinic acid in rat striatum and hippocampus. E J Neurosci 3, 40-46

Vogel CM, Drury I, Terry LC et al (1991) Myoclonus in adult Huntington's disease. Ann Neurol 29, 213-215

Vonsattel J-P, Myers RH, Stevens TJ et al (1985) Neuropathological classification of Huntington's disease. J Neuropath Exp Neurol 44, 559-577

Waeber C, Palacios JM (1989) Serotonin-1 receptor binding sites in the human basal ganglia are decreased in Huntington's chorea but not in Parkinson's disease: a quantitative in vitro autoradiographic study. Neurosci 32, 337-347

Walsh FX, Bird ED, Stevens TJ (1982) Monoamine transmitters and their metabolites in the basal ganglia of Huntington's disease and control postmortem brain. In: Gilles de la Tourette Syndrome 35, 165-169 (eds. Friedhoff AJ, Chase TN) Raven, New York

Walters AS, McHale D, Sage JI et al (1990) A blinded study of the supressibility of involuntary movements in Huntington's chorea, tardive dyskinesia, and L-Dopa-induced chorea. Clin Neuropharmacol 13, 236-240

Warsh JJ, Politsky JM, Li PP et al (1991) Reduced striatal (3H) inositol 1,4,5-triphosphate binding in Huntington's disease. J Neurochem 56, 1417-1422

Wastek GJ, Stern LZ, Johnson PC et al (1976) Huntington's disease: Regional alterations in muscarinic cholinergic receptor binding in human brain. Life Sci 19, 1033-1040

Wastek GJ, Yamamura HI (1978) Biochemical characterisation of the muscarinic cholinergic

receptor in human brain: Alterations in Huntington's disease. Mol Pharmacol 14, 768-780

Wasynczuk A, Kirksey A, More DM (1983) Effects of maternal vitamin B6 deficiency on specific regions of developing rat brain: the extrapyramidal motor system. J Nutr 113, 746-754

Waters CM, Peck R, Rossor MN et al (1988) Immunocytochemical studies on the basal ganglia and substantia nigra in Parkinson's disease and Huntington's chorea. Neurosci 25, 419-438

Watkins JC, Olverman HJ (1987) Agonists and antagonists for excitatory amino acid receptors. Trends Neurosci 10, 265-272

Watkins JC, Evans RH (1981) Excitatory amino acid transmitters. Ann Rev Pharmacol Toxicol 21, 165-204

Weinberger DR, Berman KF, Iadrola M et al (1988) Prefrontal cortical blood and cognitive function in Huntington's disease. J Neurol Neurosurg Psychiat 51, 94-104

Weissman AD, Tsung-ping S, Hedreen JC et al (1988) Sigma receptors in post-mortem human brains. J Pharmacol Exp Therap 247, 29-33

Wexler NS, Young AB, Tanzi RE et al (1987) Homozygotes for Huntington's disease. Nature 326, 194-197

Wexler NS, Rose EA, Houseman DE (1991) Molecular approaches to hereditary diseases of the nervous system: Huntington's disease as a paradigm. Ann Rev Neurosci 14, 503-529

Whetsell VO (1984) The use of organotypic tissue culture for the study of amino acid neurotoxicity and its antagonism in mammalian CNS. Clin Neuropharmacol 7, 248-250

Whetsell WO, Schwarcz R (1989) Prolonged exposure to submicromolar concentrations of quinolinic acid causes excitotoxic damage in organotypic cultures of rat corticostriatal system. Neurosci Lett 97, 271-275

Whitehouse PJ (1986) The concept of subcortical and cortical dementia: Another look. Ann Neurol 19, 1-6

Wieloch T (1985) Hypoglycaemic-induced neuronal damage prevented by an N-methyl-D-aspartate antagonist. Science 230, 681-683

Wiklund L, Toggenburger G, Cuenod M (1982) Aspartate: Possible neurotransmitter in cerebellar

climbing fibres. Science 216, 78-79

Wirsching BA, Beninger RJ, Jhamandas K et al (1989) Kynurenic acid protects against the neurochemical and behavioural effects of unilateral quinolinic acid injections into the nucleus basalis of rats. Behav Neurosci 103, 90-97

Wolfensberger M, Amsler U, Cuenod M et al (1983) Identification of quinolinic acid in rat and human brain tissue. Neurosci Lett 41, 247-252

Wong PT-H, McGeer PL, Rossor M et al (1982) Ornithine aminotranferase in Huntington's disease. Brain Res 231, 466-471

Wood PL, Emmett MR, Rao TS (1989) In vivo modulation of the N-methyl-D-aspartate receptor complex by D-serine: Potentiation of ongoing neuronal activity as evidenced by increased cerebellar cyclic GMP. J Neurochem 53, 979-981

Yates CM, Magill BEA, Davidson D et al (1973) Lysosomal enzymes, amino acids and acid metabolites of amines in Huntington's chorea. Clin Chim Acta 44, 139-145

Yates CM, Butterworth J, Tennant MC et al (1990) Enzyme activites in relation to pH and lactate in postmortem brain in Alzheimer-type and other dementias. J Neurochem 55, 1624-1630

Yeh JK, Brown RR (1977) Effects of vitamin B6 deficiency and tryptophan loading on urinary excretion of tryptophan metabolites in mammals. J Nutr 107, 261-271

Yess N, Price JM, Brown RR et al (1964) Vitamin B6 depletion in man: Urinary excretion of tryptophan metabolites. J Nutr 84, 229-236

Yong S, Lau S (1979) Rapid separation of tryptophan, kynurenines and indoles using reversed-phase high-performance liquid chromatography. J Chromatog 175, 343-346

Young AB, Penney JB, Starosta-Rubinstein S et al (1986) PET scan investigations of Huntington's disease: Cerebral metabolic correlates of neurological features and functional decline. Ann Neurol 20, 296-303

Young AB, Greenamyre JT, Hollingsworth Z et al (1988) NMDA receptor losses in putamen from patients with Huntington's disease. Science 241, 981-983

Young AB (1991) Abstract: World Fed Neurol Research Group on Huntington's chorea

Young SN, Joseph MN, Gauthier S (1983) Studies on kynurenine in human cerebrospinal fluid: Lowered levels in epilepsy. J Neural Trans 58, 193-204

Zalneraitis EL, Landis DMA, Richardson EP et al (1981) A comparison of astrocytic structure in cerebral cortex and striatum in Huntington's disease. Neurology 31, 151

Zanglein JP, Buldauf E, Hussaini M (1978) A new treatment of Huntington's chorea: Report of three cases. Sem Hop Paris 54, 871-874

Zhu SG, McGeer EG, McGeer PL (1990) γ-glutamyltransferase activity is unchanged in acutely quinolinate-lesioned rat neostriatum but is elevated in Huntington's disease caudate. Exp Neurol 107, 106-109

Zweig RM, Ross CA, Hedreen JC et al (1989) The neuropathology of aminergic nuclei in Alzheimer's disease. Ann Neurol 24, 23-242